

THE DEPOSIT OF AQUEOUS SOLUTIONS  
AND OF OIL SPRAYS<sup>1, 2</sup>W. M. HOSKINS<sup>3</sup> AND Y. BEN-AMOTZ<sup>4</sup>

## INTRODUCTION

THE VALUE OF A SPRAY OIL as an insecticide is largely dependent upon the amount of oil deposited when an emulsion containing the oil is sprayed upon an insect or upon the surface of a plant infested by the insect. Care regarding the quality of the oil, uniformity of the emulsion, and thoroughness of application is obviously of little use if the deposition of oil over the sprayed surface is insufficient, excessive, or uneven.

Previous workers who have studied the behavior of spray-oil emulsions have laid emphasis upon various properties, such as surface tension of the aqueous phase, interfacial tension between the two phases, stability of the emulsion, size of the oil droplets, angle of contact formed when the emulsion or its aqueous phase is placed upon a solid, and other analogous properties. The experiments which have been in progress in this laboratory for several years have led to the opinion that the chief importance of these properties for the deposit of oil lies in their effects upon the relative ease with which the aqueous and the oil phases make and maintain contact with the surface sprayed. During the application of an oil emulsion to a solid, and for some time thereafter, there is competition for room upon the surface of the solid. If the aqueous phase either makes contact everywhere first or is able to displace oil which has reached the surface, the end result will be little or no deposit of the oil. On the other hand, if all the oil makes contact as fast as the emulsion is applied, the deposit will be pro-

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<sup>2</sup> This article is the fourth of a series having the general title: *Factors Concerned in the Deposit of Sprays*. For earlier numbers see Hensill and Hoskins (1935); Hoskins and Wampler (1936); Ben-Amotz and Hoskins (1937) in "Literature Cited" at the end of this paper.

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portional to the duration of spraying; and excessive or deficient amounts will be left on various regions according to how the spray is applied. The desired condition lies between these extremes, and the possibility of obtaining a satisfactory deposit of oil depends upon the correct adjustment of the ability of each phase to make the necessary contact with the solid which is sprayed. The object of the work to be reported here was to acquire information concerning the effects which certain water-soluble substances have upon the behavior of the aqueous and oil phases of spray emulsions as they are applied to the standard surface of beeswax which has been used in previous studies in this laboratory (Hensill and Hoskins, 1935; Ben-Amotz and Hoskins, 1937).<sup>5</sup>

The theory that during the application of an oil emulsion each liquid phase is acted upon by forces which result in displacement of one by the other to varying degrees and that the final amount of oil left as a deposit upon the surface is a resultant of the action of all the components of the emulsion has not been stressed by either physicists or entomologists who have worked with oil emulsions. More particularly, the behavior of the nonoil components of emulsions during the act of spraying has been neglected. Since the final result is necessarily influenced by the behavior of each part of the emulsion, a desirable means of study is the examination of the separate components under conditions which resemble as nearly as possible those which prevail during application of the whole emulsion. Such a plan was followed in the present work.

This paper includes an account of what happens to the water and the emulsifying and wetting agents in oil emulsions as well as to the oil itself during and shortly after spraying. From this information, an attempt is made to interpret oil deposit in terms of certain properties of the components taken both separately and together.

For both theoretical and practical reasons, measurements of the properties of spray liquids under static conditions are of little value as compared with measurements made under the conditions which prevail during application of the sprays, that is, under dynamic conditions. This principle cannot be consistently followed in studying all the properties of sprays: for example, no truly dynamic method for measuring the stability of emulsions is available as yet. As far as possible, the experiments with spray liquids reported in this paper have been conducted under conditions which at least approximate those of actual use. In certain cases, the corresponding behavior under static conditions was studied for comparison.

<sup>5</sup> See "Literature Cited" at the end of this paper for complete data on citations, which are referred to in the text by author and date of publication.

The general scope of the investigation covered the following points: behavior of water and of aqueous solutions of the accessory substances when sprayed upon a surface, the amount of each deposited, the amount of accessory substances deposited, their distribution in the system, the angle of contact during spraying and at rest, the effect of rolling, the behavior of oil emulsions when sprayed, the wetting and spreading of the two phases, the effects of competition for space upon the solid surface, and other factors influencing the amount of oil deposited.

### THEORIES OF BEHAVIOR OF LIQUID SPRAYS

The theoretical basis for interpreting the behavior of two liquids sprayed together upon a solid surface lies in the magnitudes of the various interfacial energies and in the mechanical effects resulting from violent impact of the liquids upon the solid. The behavior of a liquid upon a solid horizontal surface has been discussed in detail by so many writers—for example, Rideal (1926) and Adam (1930)—that only certain special features need to be mentioned here. In applying an insecticidal or fungicidal spray, the intent is to reach all parts of the solid surface concerned; as far as this objective is achieved, the surface, at least while the spraying continues, becomes covered with a film of the liquid.

If the liquid is either a pure compound or a solution, its behavior after spraying has ceased depends upon whether the sum of the liquid-surface energy,  $\gamma_l$ , and the solid-liquid interfacial energy,  $\gamma_{ls}$ , is smaller or larger than the solid surface energy,  $\gamma_s$ . If  $\gamma_l + \gamma_{ls} < \gamma_s$ , the liquid will remain in a film over the surface of the solid; but if  $\gamma_l + \gamma_{ls} > \gamma_s$ , it will recede from the surface and gather into drops, whose area will be subject to the equilibrium condition that:

$$\gamma_s = \gamma_{ls} + \gamma_l \cos \theta, \quad 1$$

in which  $\theta$  is the equilibrium angle enclosed by the liquid at the edge of the drop. In spraying natural surfaces, variations in behavior are often noted between different regions of the same leaf or fruit. These are sometimes due to real differences in the nature of the surface at different points and sometimes to the presence of foreign material, such as dust. If finely divided material which is wet but poorly by the spray is on the surface, real contact may not be made with the surface; and the liquid will retreat very rapidly when spraying has ceased. In the special case when  $\gamma_l + \gamma_{ls} = \gamma_s$ , the extent to which the liquid film extends will be particularly sensitive to mechanical disturbances. This condition is likely to occur only in certain regions rather than over any entire natural surface.



The introduction of a second liquid phase into the spray leads to greater or less contact of the new liquid with the solid surface and hence to competition for space between the two liquids and ultimately to partial or complete replacement of one liquid by the other. The energies concerned in these processes are those residing in the surfaces of the two

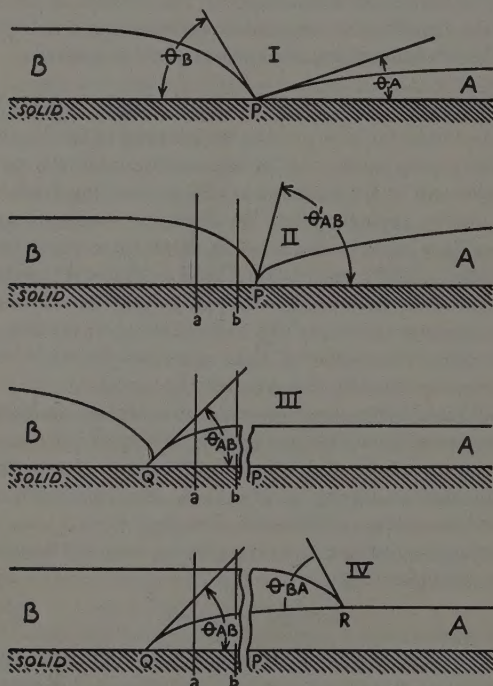


Fig. 1.—Displacement of one liquid from a solid surface by a second liquid.

liquids, in the interface between the liquids, and in the interfaces between each liquid and the solid. In figure 1 are shown diagrammatically certain of the situations which have been observed when limited volumes of oil A, and aqueous solution B, were applied gently to adjacent areas of a surface. In section I, the advancing front of liquid A, which makes a small contact angle,  $\theta_A$ , upon the solid S, meets liquid B, which is advancing in the opposite direction and which makes a larger contact angle  $\theta_B$ . In section II, the depth of both liquids at the line of contact has increased,

and the interface between the two liquids has been established so that it makes the angle  $\theta'_{AB}$  with the solid surface. This usually is not the equilibrium angle, and the system will change to a condition such as III or IV. Since liquid *B* has been replaced by liquid *A*, the changes in energy per square centimeter of solid surface in III, as in region *a-b*, are  $(\gamma_A + \gamma_{SA}) - (\gamma_B + \gamma_{SB})$ , which may be called the "replacement coefficient" for any case in which one liquid is pushed back by another and the one which is replaced does not spread over the other. Since limited volumes were assumed for each liquid, obviously the one which spreads will soon be in a very thin layer while the thickness of the other will become greater. At length an equilibrium will be reached whose geometrical characteristics will depend on whether contact is made upon a flat surface, within a trough, within a capillary tube, etc., but which will be characterized by an equilibrium angle  $\theta_{AB}$  between the solid and the plane of contact of the two liquids where it meets the solid as at point *Q*. When equilibrium has been established, the relations at the line of maximum extension of liquid *A* will be expressed by the equation:

$$\gamma_{SB} = \gamma_{SA} + \gamma_{AB} \cos \theta_{AB}. \quad 2$$

The situation in which the displaced liquid spreads over the other is represented in section IV of figure 1. The energy change per unit area is  $\gamma_B + \gamma_{AB} + \gamma_{SA} - (\gamma_B + \gamma_{SB})$ , or  $\gamma_{AB} + \gamma_{SA} - \gamma_{SB}$ . The equilibrium condition will satisfy equation 2 and  $\theta$  will have the same value as  $\theta$  in III unless the layers of liquid are so thick that gravity has an appreciable effect. Liquid *B* may not spread entirely over liquid *A* but instead may assume an equilibrium angle,  $\theta_{BA}$ , upon it. The equilibrium conditions at *R* may be expressed by the same kind of equation as for a liquid spreading upon a solid, in this case:

$$\gamma_A = \gamma_{AB} + \gamma_B \cos \theta_{BA}. \quad 3$$

Only under certain conditions is the above discussion rigidly valid for the behavior of an oil emulsion applied to a solid. The more important of these conditions are: (a) the surface is horizontal; (b) the effects of gravity are negligible; (c) the force with which the liquids strike the surface can be neglected; and (d) the effects due to emulsifying and wetting agents present in the emulsion are those for equilibrium conditions. Obviously in many situations none of these limitations hold. The numerous discrepancies between theory and fact in the use of sprays are largely due to the false assumption that an ideal system is being studied. In later sections, the practical effects of certain of the factors listed above will be discussed in detail.

In a system involving water, oil, a solid, and a soluble surface-active substance, the last-named may obviously exert its effect at any or all three of the possible interfaces—(a) water-oil, (b) water-solid, and (c) oil-solid. As a matter of fact, any actual substance of this nature tends to collect to varying extents at all three interfaces, but a distinction may be drawn between *emulsifying agents*, which primarily affect interface *a*, and *wetting and spreading agents*, whose chief effect is upon interfaces *b* and *c*.

The difference between wetting and spreading agents has been discussed by numerous workers, but the terms have not always been distinguished clearly. The various points concerned are covered satisfactorily in the following three sets of definitions: (1) A wetting agent is "any substance which causes a spray fluid to wet the sprayed surface so that 'running up' into drops on the surface is avoided;" a spreading agent is "any substance which tends to cause lenses of spray to spread over those portions of the plant surface which have not been hit directly by the spray" (Woodman, 1930). (2) "A wetting agent is any substance which increases the readiness with which a liquid makes real contact with a solid;" "a spreader is a material which increases the area that a given volume of liquid will cover on a solid or another liquid" (Hensill and Hoskins, 1935). (3) "Wetting properties are defined by the ability to form a persistent liquid-solid interface when excess of liquid is drained from the surface;" "spreading properties are defined by the ability of the liquid to form a liquid-solid interface solely by surface activity over the plane surface of the solid" (Evans and Martin, 1935). In order to avoid the use of long and awkward expressions in referring to materials added to the oil-water system for the purpose of altering its properties in one way or another, the term "accessory substance" will be used when no particular function is being emphasized.

The oil-water interface may be affected by a third component which is predominantly soluble either in oil or in water. Hence, both the stability of an emulsion and the relative ease of contact of oil and water with a solid surface may be affected by both water-soluble and oil-soluble accessory substances. The present work has been limited to those which are predominantly water-soluble. Chemically speaking, such substances will have an affinity for water, that is, the phase in which they are more highly soluble, but in order to be able to concentrate in the water-oil interface, they must possess a chemical group or groups which are soluble in the latter also. All emulsifying agents have an affinity for each of the liquid components of the emulsion. Similarly, a substance which aids a liquid to wet a solid must contain groups which have an affinity for



both the liquid and the solid, respectively. Since solids may or may not be similar in constitution to oils, a substance which is a good emulsifying agent for hydrocarbon oils will have different effects in promoting the wetting of various solids by water. A high emulsifying power is not necessarily accompanied by high wetting power and vice versa. Thus, proteins are effective wetting agents for beeswax surfaces, but are relatively poor emulsifiers for hydrocarbon oils, while soaps possess these properties in the reverse relation.

The extent to which a substance may effect the emulsifying, wetting, or spreading properties of a liquid or mixture of mutually insoluble liquids, such as an oil-water emulsion, is dependent upon the time allowed for it to migrate into the interfaces concerned; for the over-all concentration in the bulk of the liquid is usually low, and the marked effects exerted by surface-active substances are due to their ability to become concentrated in regions of transition from one kind of matter to another. The importance of time as a factor in the behavior of oil sprays was emphasized by Ben-Amotz and Hoskins (1937) and will be further illustrated in subsequent sections of this paper.

#### MATERIALS, APPARATUS, AND METHODS

Three water-soluble surface-active materials, a soap, blood albumin, and hemoglobin, were used as accessory substances. The soap was a so-called "neutral" powder, U.S.P. sodium oleate. It was finely divided, slightly yellow in color, and dissolved completely to give a practically colorless opalescent solution. A stock solution of known strength, made up fresh from time to time, was used in preparing the various solutions and emulsions.

The blood albumin was a commercial preparation of dried blood proteins known as Mapco Blood Albumin. It was a brownish-red powder, containing approximately 3 per cent of insoluble material. A stock solution was used for preparing the various dilutions.

The hemoglobin was prepared in the laboratory from fresh beef blood according to the method of Morse (1927). Great care was taken to avoid conditions likely to cause chemical or physical changes in the hemoglobin, and the various preparations were kept at 0° C in aqueous solution. The concentration of each stock solution was determined by analysis for nitrogen and for iron. In each case the two methods agreed within 1 per cent.

The accessory substances were used at concentrations varying from zero to 0.0225 per cent by weight. For convenience, actual measurements were made in milligrams per gallon of spray and are so given in the

graphs. The relations of milligrams per gallon, per cent by weight, and ounces per 100 gallons are as follows:

Milligrams per gallon	Per cent by weight	Ounces per 100 gallons
71	0.0019	$\frac{1}{4}$
142	0.0037	$\frac{1}{2}$
284	0.0075	1
568	0.0150	2
852	0.0225	3

The oil used in all experiments was a "white neutral" tank-mix stock oil of 80 seconds Saybolt viscosity, 92 per cent unsulfonatable residue, and 0.85 grams per cc density. It was secured from the refinery at intervals and kept in closed cans to minimize the changes that take place during storage. This oil was used at 2 per cent by volume in all the emulsions.

The water was ordinary Berkeley tap water whose pH is 8-9 and degree of hardness 45-50 p.p.m.  $\text{CaCO}_3$ . The temperature of the spray liquids when ready for use was 16-20° C.

The surface to which the sprays were applied was a commercial white beeswax, which, for application, was dissolved in carbon tetrachloride at 35° C to a concentration of 4.5 per cent by weight. Oil sample bottles 13.6 cm in height and 3.6 cm in diameter (total lateral area 153 sq. cm) and glass plates  $3\frac{1}{4}$  by  $4\frac{1}{4}$  inches were dipped in the wax solution and then set on a rack to dry for 24 hours. A very thin hard coating was left which appeared to be uniform in properties and whose weight was constant thereafter. The bottles and plates were used as soon thereafter as possible, for after a few days the surface became split by fine cracks and behaved differently when sprayed.

*Measurements Made under Static Conditions.*—Surface tensions were determined with the du Noüy interfacial tensiometer. All measurements were made by pulling the ring upward. In preparing for a test, care was taken to disturb the surface to a minimum extent in order that the experimental result might be an accurate measure of the tension of a surface of the given age.

*Angle of contact* was determined by placing a small drop of the liquid upon a horizontal waxed plate and photographing it from the side.

*Replacement of one liquid by another* was studied by placing drops of each very close together and visually following the changes in position after the drops touched.

*Emulsifying power* was studied by agitating for 1 minute with a milkshake mixer equal volumes of oil and a solution of the chosen accessory substance. The emulsions were then allowed to stand in tall cylinders



and the time required for 50 per cent of the oil to escape and collect at the top was determined.

*Measurements Made under Dynamic Conditions.*—The extent of wetting of a surface as spray is applied to it was determined by taking photographs with 0.001 second exposure (Craig, 1936). By doing this at intervals, the sequence of events can be followed as a spray is applied.

The shape assumed by drops as they roll down a vertical or inclined surface was also studied by the photographic method.

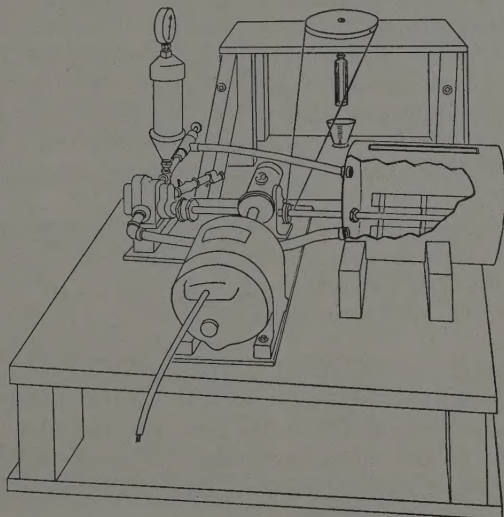


Fig. 2.—Apparatus used in spraying.

The important distinction between the *advancing* and *retreating angles of contact* made by rolling drops is brought out clearly by photographs.

The spraying apparatus consisted of a small gear pump attached to a horizontal 2-gallon tank equipped with an agitator having long flat blades of the ice-cream-freezer type, which revolved 240 times per minute. The nozzle was of the Vermorel design with a disk opening  $\frac{1}{32}$  inch in diameter. By properly setting the pin, a solid cone of spray of uniform density can be secured. The objects to be sprayed were placed in a hood 55 cm distant from the nozzle and centered in the spray cone. The bottles were attached by means of a cork to the lower end of a vertical iron rod which rotated at 15 r.p.m. The entire apparatus is shown diagrammatically in figure 2.

The sprays were prepared by putting 1 gallon of water in the tank with the required amount of accessory substance. The agitator was set in motion and oil in the standard amount of 2 per cent by volume was added. After agitation had continued for 5 minutes, the pump was started and the emulsion was recirculated to the tank under a pressure of 80 pounds to the square inch for 1 minute. Sufficient spray was then wasted to flush out the pipes and the waxed bottles were sprayed as rapidly as possible.

The volume of spray applied to each bottle was regulated by catching the runoff in a funnel and graduated tube set closely beneath the bottle. Spraying was continued until the volume of the liquid was 20 cc. At a pressure of 80 pounds per square inch very little liquid is knocked off the bottle, and consequently that which drains from the bottom is a reliable index of the total volume which struck the surface. By means of a shutter set in front of the nozzle, the volume of the spray which reaches the bottle can be controlled to within 1 cc. The corresponding variations in deposit are negligible.

Four bottles were sprayed for each deposit test, and the tests were repeated several times for each condition.

The amount of oil deposited was determined by the difference in weight before and after spraying, 48 hours being allowed for all water to evaporate.

The amount of aqueous solution or of entire emulsion deposited was determined also by the difference between weights before and after spraying, but in these cases the bottles were taken from the spray chamber, placed for 5 minutes in a compartment saturated with water vapor in order that excess liquid might drain off, and then weighed as rapidly as possible.

For studying the amount of accessory substance left upon the surface under various conditions, hemoglobin was used and estimated by analysis for nitrogen by a micro-Kjeldahl method.

## RESULTS

*Effects of the Accessory Substances upon the Wetting and Depositing Powers of the Aqueous Phase.*—The study of the separate components of the emulsions was begun with the aqueous phase since it is the only one in which the surface-active added substances are soluble and which, therefore, may be expected to reveal most simply the effects of their presence.

In figure 3, *A*, the behavior of water and of solutions of blood albumin, hemoglobin, and sodium oleate during application to waxed bottles is shown by photographs taken with 0.001 second exposure according to the method of Craig (1936). When the pictures were taken, spraying had



Fig. 3.—*A*, Behavior of aqueous solutions of the accessory substances during spraying; *B*, behavior of drops of aqueous solutions of the accessory substances as they move down an inclined wax surface. Composition of the solutions: *a*, water; *b-d*, blood albumin; *e-g*, hemoglobin; *h-j*, sodium oleate. Each of the accessory substances is in a series of increasing concentrations—35, 142, and 568 mg per gallon.



been in progress for 10 seconds, during which time approximately half the standard runoff of 20 cc had occurred. The appearance at corresponding concentrations of the two protein solutions is somewhat similar and is in sharp contrast to that prevailing with the soap. At equal concentrations, hemoglobin gives somewhat better wetting than the commercial blood albumin. The lowest concentration of each substance causes the solutions to wet but little better than water; but thereafter the proteins cause the formation of broad shallow drops, which coalesce to run down in streams. With the highest concentration illustrated (568 mg per gallon), wetting is fairly well accomplished and broad sheets cover a considerable portion of the area. The soap solution at the highest concentration shown forms only a few narrow streams and no sheets. As a matter of fact, comparable wetting scarcely occurs with solutions of sodium oleate six times as concentrated as those of the proteins.

The contrast between the effects of the two types of accessory substances is illustrated further by photographs taken several minutes after spraying was completed. These conditions are shown in figure 4. The transition from small hemispherical drops to broad shallow drops and eventually to complete coverage of the wax surface by a continuous sheet of solution is shown clearly for the two proteins. On the other hand, even the highest concentration of soap causes only limited formation of streams and no continuous coverage.

The observations and photographs made both during and after spraying leave no doubt that as measured under actual conditions of use, soap solutions are very poor wetting agents for beeswax surfaces, whereas the two protein solutions, particularly the hemoglobin, are excellent for that purpose.

A quantitative study of the amounts of solution left upon the surface was made by allowing each tared bottle to drain for 5 minutes in a chamber practically saturated with water vapor and then weighing it. Very little drainage occurred after this interval.

The results are given in figure 5. They bring out the important fact that whereas the formation of shallow drops tends to increase the amount of liquid remaining upon the surface, as soon as conditions are such that complete wetting occurs, the amount remaining is very greatly reduced. This result is in accord with the finding of Evans and Martin (1935) that the better the wetting the less the maximum amount of spray that could be applied before runoff occurred. Although the conditions of their work and those of the present experiment were very different, in both cases improvement in wetting led to decreased deposit. The soap solution, which does not pass through the state of forming shallow drops but

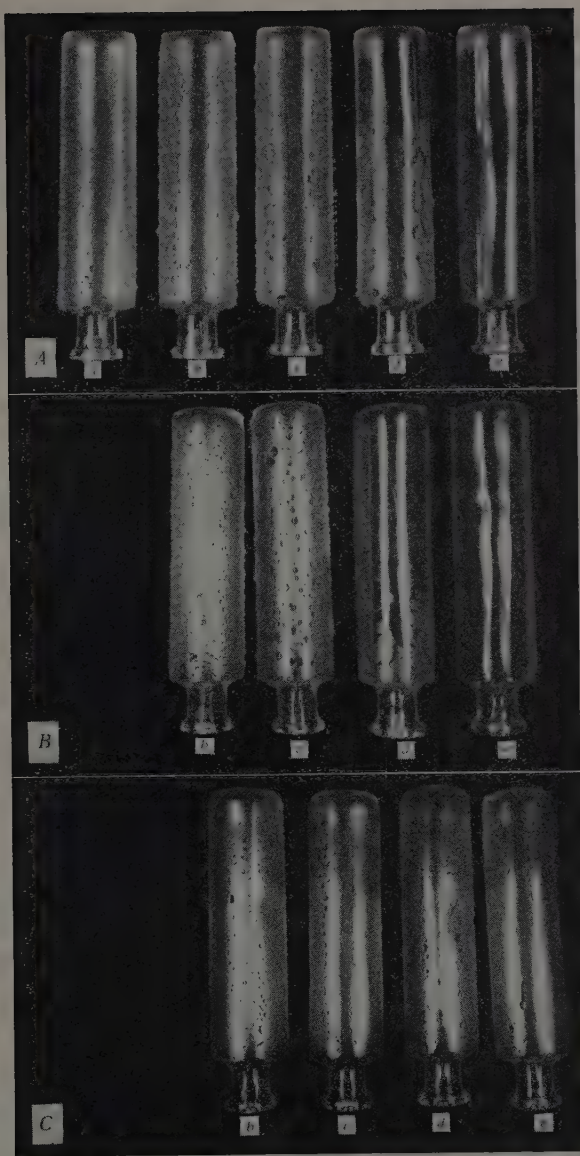


Fig. 4.—Appearance of bottles several minutes after spraying with aqueous solutions of the accessory substances: *A*, *a*, water; *b*–*e*, blood albumin; *B*, hemoglobin; *C*, sodium oleate. Each of the accessory substances is in a series of increasing concentration—*b*, 71 mg per gallon; *c*, 142 mg per gallon; *d*, 284 mg per gallon; *e*, 568 mg per gallon.

forms very narrow streaks instead, remains upon the surface in continuously decreasing amounts as more solute is used.

It was very easy to notice during the act of spraying that the various solutions made widely differing angles of contact with the solid; to some extent these effects can be seen in the photographs of figure 3, *A*. In order to study this behavior to better advantage, photographs with 0.001-sec-

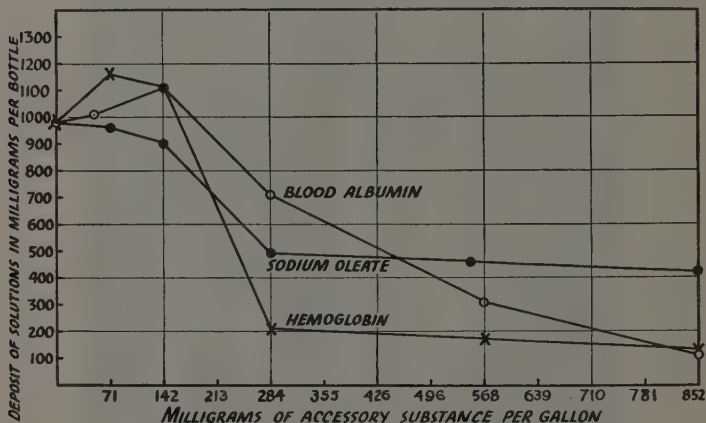


Fig. 5.—Total deposit of solutions of the various accessory substances.

ond exposure were taken of drops of the solutions as they rolled down a waxed slide set at an angle of  $24^{\circ}$  to the vertical.

The results are shown in figure 3 *B*. Since the drops were made to fall very rapidly from the tip of the burette, they were of nearly uniform size, and several could be photographed at once as they moved down the slide. The following significant differences in behavior of the solutions may be noted: moving drops of the water and of all the soap solutions assume approximately the same shape; but with successively higher concentrations of protein, drops of these solutions flatten, and with the greatest amounts present (fig. 3 *B*, *d* and *g*), a continuous sheet so thin that it cannot be seen from the side is formed by coalescence of the successive drops.

A somewhat similar method of studying spray liquids was suggested by Heranger (1936) who allowed drops to move down the underside of an inclined tube. Under those conditions, the drops are distorted on account of the shape of the solid. For this reason, the present method seems to approximate more closely conditions of normal use of sprays.



The difference between the advancing and retreating angles established during movement of drops down a wax surface is brought out clearly in figure 3 *B*. In the case of water and the soap solutions, the advancing angle is somewhat greater than  $90^\circ$ , whereas the retreating angle is considerably less. However, the important point is not the precise magnitude of these angles but the fact that the latter have a finite magnitude, that is, the drops move as a whole and do not leave a layer of solution behind them. By contrast, the protein solutions, particularly that of hemoglobin, form long tails and, with the higher concentrations, spread from one drop to the next and make a zero retreating angle with the wax surface.

Since this ability of the protein solutions to adhere strongly to the surface is entirely due to the solute, it was of great interest to learn if the proteins become concentrated upon the wax. This was done in the following manner: bottles were sprayed in the usual way, taken from the stand, and shaken several times very vigorously. By this procedure most of the liquid was mechanically removed, and a fairly uniform weight of about 125–150 mg was left as a thin film in all cases. The amounts of protein in these strongly adherent portions of the spray solutions, as calculated from micro-Kjeldahl determinations of nitrogen, are given in columns 2 and 3 of table 1. From the original concentrations in the spray solutions, the amounts of protein in this weight of solution may be calculated approximately, by assuming unit density, to vary from 0.0025 to 0.031 mg. Upon dividing the first and last numbers in columns 2 and 3 of table 1 by these figures, it is found that the blood albumin is from 60 to 25 times as concentrated in the strongly adherent film upon the bottles as in the original solutions and the hemoglobin from 140 to 20 times as concentrated, depending upon whether the weakest or the strongest solutions are compared. As is usual with adsorption processes, the greater relative effect is found with the more dilute solutions.

To gain further information regarding the collection of protein at the wax-liquid interface, analyses were made to determine the amount of blood albumin in all the solutions which remained upon the bottles after spraying, that is, those shown in figure 4. The amounts are given in column 4 of table 1. Comparison of columns 3 and 4 shows that most of the protein remained upon the bottles when they were shaken, though for the lower concentrations at least 80 per cent of the adhering liquid was shaken off. Hence, this experiment also indicates that the protein accumulated at the solid-solution interface.

On account of the emphasis placed by certain writers upon the importance of surface tension as a measure of the wetting or spreading

properties of spray solutions, determinations were made of the surface tensions of the various solutions of blood albumin, hemoglobin, and sodium oleate used in the spraying experiments. All measurements were made upon surfaces 15 seconds old since this is about the least time in which trustworthy tests can be carried out with the du Noüy instrument.

The results are given in table 2. Apparently the differences in behavior of the various solutions during spraying cannot be correlated with the surface tension measured in this way. The tension of older surfaces, such

TABLE 1  
WEIGHTS OF PROTEINS IN SPRAY REMAINING UPON WAXED BOTTLES

Concentration of accessory substance in spray liquid	Weight of protein on bottle			
	From solution			From emulsion (not shaken): hemoglobin
	After shaking		Not shaken: blood albumin	
	Hemoglobin	Blood albumin		
1	2	3	4	5
mg per gal.	mg	mg	mg	mg
71.....	0.35	0.15	0.25	0.22
142.....	0.41	0.31	0.41	0.23
284.....	0.47	0.47	0.66	0.26
568.....	0.54	0.63	1.14	0.30
852.....	0.59	0.70	0.70	0.34

as are ordinarily used for such measurement, are even less likely to be related to the wetting power of spray liquids during application.

*Effects of the Accessory Substances upon the Behavior of the Oil Phase and of the Complete Emulsion.*—The introduction of oil into an aqueous spray liquid leads at once to the situation previously described, that is, competition between the two phases for room upon the surface sprayed. The extent of separation of oil and water during the time an emulsion is passing through the air from nozzle to surface is apparently not known; but since this period is very short with the arrangement used in the present work, most of the surface of the spray drops is probably aqueous. Accordingly, after impact of the spray, there is opportunity for migration of the accessory substance to the solid-aqueous interface, and wetting occurs to an extent dependent upon the concentration of the wetting agent and the nature of the solid.

Photographs of the behavior of various emulsions during spraying are shown in figure 2 of the previous article in this series (Ben-Amotz and Hoskins, 1937). Comparison of these pictures with those for the solutions

containing no oil (fig. 3, A, of this paper) reveals that the emulsions wet the surface less easily than the corresponding solutions of the accessory substances. When the aqueous phase wets poorly—for example, in the absence of accessory substances or with soap solutions—the droplets grow in size upon the surface during spraying until under the influence of gravity, they begin to move downward. Their shape is similar to that

TABLE 2  
SURFACE TENSION\* OF SOLUTIONS OF THE VARIOUS  
ACCESSORY SUBSTANCES  
(All surfaces were 15 seconds old)

Accessory substance	Concentration	Surface tension, $\gamma$
	<i>mg per gal</i>	<i>dynes per cm</i>
None.....	0 (tap water)	74.0
Blood albumin.....	{ 71	65.0
	{ 142	62.6
	{ 284	59.5
	{ 568	57.5
	{ 852	56.4
Hemoglobin.....	{ 71	70.5
	{ 142	67.4
	{ 284	64.4
	{ 568	61.6
	{ 852	53.8
Sodium oleate.....	{ 71	69.9
	{ 142	66.6
	{ 284	63.5
	{ 568	58.9
	{ 852	49.1

\* These data are as read on the du Noüy tensiometer. To reduce them to absolute values of  $\gamma$ , multiply by factor 0.943, calculated by method of Harkins and Jordan (1930).

shown in figure 3, B, a, h, i, and j. At no time is there a large area of contact between the emulsion and the surface, and consequently a comparatively small portion of the available oil ever reaches the solid. If a drop of such an emulsion is allowed to move down the surface of a waxed slide or bottle, the oil which separates upon the solid can be seen as isolated spots. Complete coverage can be secured only by allowing many such drops to roll down a given portion of the surface.

When the aqueous phase has moderately good wetting power—for example, with the protein solutions containing 35 to 142 mg per gallon—the drops of emulsion elongate as they move and form long tails in which the oil droplets congregate. Opportunity is thus afforded for oil to deposit upon the surface if it is able, first, to make real contact, and second, to



displace the aqueous phase. As far as these actions occur, the surface changes from the original solid wax to liquid oil. An oil surface is wet by protein solutions much less readily than is wax. For this reason, the emulsions have more difficulty in remaining extended upon the surface as the latter is progressively converted to oil. At some characteristic concentration for each protein, conditions will be such that maximum oil will reach the sprayed surface. With higher concentrations, the aqueous phase has so much wetting power that oil has excessive difficulty in displacing it from the surface; hence, less oil may be expected to be deposited.

Additional information concerning the phenomena which occur during the application of emulsions to wax surfaces was secured by a study of the amounts of the various components deposited during the process of spraying. The total was determined by allowing the tared bottles to drain in the humidity chamber for 5 minutes before weighing, just as in the determination of total deposit of the solutions. The results are shown in figure 6. In this same figure are shown the amounts of oil deposited per bottle from 20 cc runoff of each emulsion. The difference between total deposit of emulsion and oil deposit is the amount of aqueous phase left upon the surface. Obviously the curves representing the deposits of the aqueous phase are in general similar in shape and close to those for total deposit.

As was mentioned above, the photographs of the spraying process show that the various emulsions wet the surface more and more poorly as the deposit of oil increases. The deposits of the entire emulsions and of the aqueous phases bear out this observation.

Thus the decided increase in total deposit with higher concentrations of blood albumin is consistent with the abrupt decrease in the amount of oil deposited, for the surface is thereby wetted more easily. At the same time, the formation of a thin film of the aqueous phase is not possible, so that no decrease in deposit occurs from that cause as occurred with the aqueous solutions alone (fig. 5).

In the case of hemoglobin, the increase in total deposit with small amounts of the protein is caused by increased ease of wetting even in the presence of the oil; but thereafter the uniformly large amount of oil on the surface causes the deposit of emulsion to remain nearly constant as the concentration of hemoglobin increases.

The nearly constant small amount of emulsion deposited in the case of sodium oleate may be explained as a consequence of the very poor wetting properties of this material over the concentration used, together with the fairly constant deposit of oil.

A comparison of figures 5 and 6 will show that with the higher concentrations of accessory substances, more of the aqueous phase containing the proteins remains upon the surface than when only the solutions are used, whereas the reverse is the case with soap. This shows clearly that

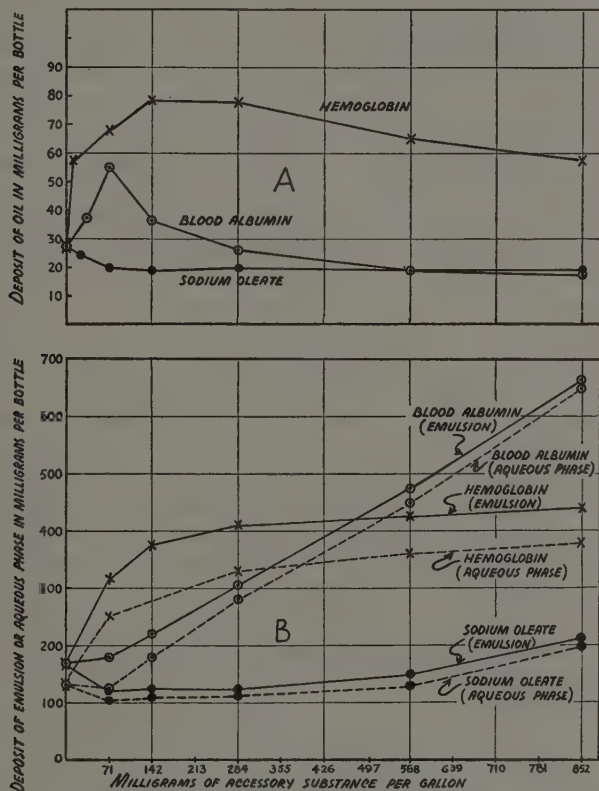


Fig. 6.—Deposit of oil, total emulsion, and aqueous phase resulting from different concentrations of accessory substances.

the greater difficulty in wetting an oil surface and consequent formation of drops instead of sheets increases the amount of the aqueous phase which adheres to the surface. Since the soap solution is not able to form continuous sheets on either wax or oil, a similar reversal would not be expected.

Further information regarding the behavior of oil emulsions during

spraying was obtained by analyses to determine the amounts of accessory substance left upon the wax surface during the process of spraying. Comparison of the data in columns 5 and 2 of table 1, shows that hemoglobin was present in amounts somewhat over half as great as when no oil was in the spray. Thus less accessory substance was deposited from the emulsion than from the aqueous solution; but since drops of the emulsion clung to the oily surface, this experiment shed no light on whether the hemoglobin was still upon the wax, that is, beneath the oil, or whether it was all in the aqueous phase. In order to determine this point, two kinds of experiments were performed.

In the first experiment, upon bottles which had been sprayed with an emulsion containing hemoglobin, the locations of the adherent drops of the whole emulsion were marked, and the spray was then allowed to dry. The spot-test reagent of Lucas (1935) was then applied to various regions of the surface. This reagent is a solution of sodium perborate and benzidine in glacial acetic acid, and it changes from pale straw color to blue in the presence of extremely small amounts of hemoglobin. The results were very clear-cut; for even the smallest drop of the whole emulsion gave an unmistakable response, but the rest of the surface gave no color. It is scarcely possible that the oil covered over any hemoglobin and prevented a reaction; for after standing several hours, as in some of these experiments, the oil enters into the wax layer and leaves the surface exposed again. Furthermore, when a drop of hemoglobin was allowed to dry upon the wax surface and a thin film of oil was added and allowed to stand for a time, the test was very positive. This experiment indicates that hemoglobin follows the aqueous phase and does not remain behind when water is displaced by oil.

The second test of this theory consisted of placing drops of oil and of hemoglobin solution adjacent to one another upon a waxed slide. The areas in which the solution was replaced by oil gave no test for hemoglobin.

No direct study of the behavior of blood albumin or of sodium oleate could be made since similar simple tests are not available. There is every reason to believe that blood albumin acts like hemoglobin. In fact, during the replacement of their solutions by oil, both proteins may be observed to pile up in front of the oil as it advances. Probably not even a unimolecular layer of the water-soluble proteins is left upon wax as the latter becomes covered by hydrocarbon oils. A definite conclusion can scarcely be drawn for soap, which has much more affinity for oil. However, the very poor wetting power of soap solutions for wax is an indication that soap also will follow the aqueous phase.



A number of experiments were made to study the displacing power of oil for the various solutions of accessory substances placed upon waxed plates. In the case of water and of any of the soap solutions within the concentration range used in the spraying work, displacement was complete. The oil crept around and beneath the aqueous drop, which eventually floated upon the oil in the form of a flattened sphere. Probably because water is heavier than oil, the latter was present beneath the drop of water only in a very thin but continuous film.

When a protein solution was used, the initial rate of displacement was slower, and equilibrium was reached in some one of the positions indicated in figure 1. The higher the protein concentration the less the replacement and the more closely the equilibrium state resembled figure 1, II. On account of its great importance in connection with all oil emulsions, the replacement of one phase by the other merits further detailed study.

### DISCUSSION

In spite of the importance of the quantity of liquid left upon a plant or other sprayed surface when an aqueous solution of an insecticide or fungicide is applied, very little information on the subject has been recorded. Emphasis is usually laid upon complete wetting of the surface; and of course the importance of reaching each part, at least during the application of the spray, cannot be denied. Unfortunately, however, as shown by Evans and Martin (1935) and in the present investigation, the state of complete wetting is also that of low retention of liquid. The discovery that dissolved proteins become concentrated in the adhering liquid suggests that toxic materials, for example, nicotine or polysulfide, may be held in a similar manner. If this should prove to be the case, then wetting agents would probably affect such behavior, and the possible helpful or harmful effects in this respect of various accessory substances would offer a fruitful field of investigation.

*Difficulties in Experimental Work.*—Efforts to gain information about the behavior of sprays during actual use must be made under two kinds of handicap. Theory has been developed extensively for static but not for dynamic conditions. At least two additional forces must be introduced into the expressions for the behavior of a liquid sprayed against a solid, that is, gravity and impact pressure. The steady increase in the pressure under which sprays are being applied as more powerful pumps are developed causes the latter factor to be of more and more importance. Until such theoretical advances are made, recourse must be had almost entirely to empirical methods. Here the second difficulty arises; for the necessary technique has been developed but slightly. The complete failure of the

drop method for measuring dynamic angles of contact was pointed out by Ben-Amotz and Hoskins (1937) and illustrated by photographs. The rotating cylinder method of Ablett (1923) is undoubtedly an improvement and merits trial with spray solutions. The ordinary method of changing the angle of incidence of a slide introduced into the liquid has been shown by Evans and Martin (1935) to give results suitable for correlation with deposit of solution formed by their special method of spraying only up to runoff, but it does not apply to the method of overspraying ordinarily used.

*Importance of Dynamic Retreating Angle and Static Advancing Angle.*—A distinction must be drawn between the situations in which the advancing angle and those in which the retreating angle is of prime importance. When the degree of wetting achieved by a liquid over the surface sprayed and the amount of the liquid retained are the chief considerations, as in the present work, the retreating angle is a controlling factor. Contact having been made with the surface by spraying under pressure, the behavior of the liquid thereafter depends upon its tendency to draw back and roll off. Contact brought about in this way may not be wetting at all in the sense of a meeting of molecules which have a marked degree of affinity for each other.

If the spray is a pure liquid or a solution, that is, consists of one phase only, the amount which will remain upon the solid will not be influenced seriously by the manner in which excess liquid leaves the surface during spraying, but it will be affected greatly by the behavior of that which is present just as spraying is stopped. For this situation, the best criterion is the retreating angle of contact measured under dynamic conditions simulating those prevailing at the time concerned. The migration of solute molecules to an interface and their orientation there are functions of time. Hence a measurement made after more or less prolonged contact, as is the usual method, does not represent the behavior of a spray in ordinary use; for drainage under the influence of gravity occurs rapidly.

The spreading of a spray beyond the limits to which it is driven during application may be of great importance, for example, in reaching beneath the covering of a scale insect or between the petals of an unfolding bud. Such extensions of a liquid usually occur rather slowly. Hence measurements of the advancing angle of contact made under static conditions may be entirely appropriate for study of such behavior.

In short, the static advancing angle is a valuable measure of the ability of a liquid to spread, and the dynamic retreating angle is an equally valuable criterion of its ability to wet. In each case the indication is valid only for the solid upon which the measurement was made, though spread-

ing is more sensitive to surface conditions than is wetting (Bartell and Wooley, 1933). The rapidity with which a liquid changes in shape or position is, of course, dependent also upon its viscosity (Woodman, 1924; Research Staff of General Electric Company, 1922).

*Effect of Stability of Emulsion.*—The foregoing discussion of the behavior of liquids applies to any single-phase spray, though, in the present work, interest was centered in the aqueous phase largely because it is the carrier for the emulsified oil. The introduction of a second, finely divided liquid, such as oil, broadens the range of variation in the system by at least three additional factors: stability of the emulsion, competition for space upon the solid, and alteration of the surface as oil increases in amount upon it. Of these, the first was discussed by Ben-Amotz and Hoskins (1937), who showed that the more stable an emulsion, other things remaining equal, the less oil will be deposited from a given volume of it. As the stability increases, the individual oil droplets make contact with the solid surface with greater and greater difficulty and fewer of them escape from the layer of emulsifying agent which has collected in the oil-water interface. The ideal method of measuring stability in this case would evaluate this ability of oil to escape from the emulsion to the solid. Such a method, separated completely from wetting phenomena of the aqueous phase, does not seem to have been developed.

The arguments advanced earlier regarding the importance of the dynamic retreating angle of contact as a factor which determines the amount of a homogeneous liquid which will remain upon a sprayed surface must be emphasized from yet another point of view in the case of emulsions. When the aqueous phase has moderately good wetting power, the drops elongate as they roll and in the tails the droplets of oil congregate. They are thereby brought close to the solid, and their chances of making contact with it are enhanced. As emphasized before, at some certain concentration of each accessory substance which allows sufficient wetting, maximum oil will be left upon the surface. This condition is realized with the two proteins but not with the soap; for solutions of the latter do not have pronounced wetting power except at concentrations so high that the deposit, because of the great stability of the emulsion, is extremely low. How much the conditions of replacement of the aqueous phase by oil are influenced by the pressure with which the spray liquid strikes the surface apparently has not been investigated. Until this information is available, experiments on replacement under static conditions cannot be used for quantitative comparison of various emulsions, but they certainly permit qualitative comparison.

*Primary and Secondary Deposit.*—The gradual alteration in the

sprayed surface from the original solid through spotted attachment and spreading of oil droplets to the final continuous layer of oil affords opportunity for division of the process into steps. The terms "primary" and "secondary" deposit were used by Smith (1933) and with slightly modified meaning by Hensill and Hoskins (1935). In the light of certain ideas developed in this report, they may be further elaborated. The primary deposit really consists of two parts. In the beginning, oil droplets are

TABLE 3  
FRACTIONAL PART OF TOTAL OIL DEPOSIT CONTRIBUTED BY SECONDARY DEPOSIT

Concentration of accessory substance	Weight of aqueous phase left			Minimum weight of oil corresponding to aqueous phase			Total oil deposit (from fig. 7)			Minimum fraction of total oil deposit from secondary deposit		
	Blood albumin	Hemo-globin	Sodium oleate	Blood albumin	Hemo-globin	Sodium oleate	Blood albumin	Hemo-globin	Sodium oleate	Blood albumin	Hemo-globin	Sodium oleate
mg per gal.	mg	mg	mg	mg	mg	mg	mg	mg	mg	per cent	per cent	per cent
0.....	140	140	140	2.4	2.4	2.4	28.0	28.0	28.0	9	9	9
71.....	125	250	155	2.1	4.2	2.6	55.0	68.0	20.0	4	6	13
142.....	180	300	165	3.1	5.1	2.8	37.0	78.5	19.5	8	6	14
284.....	280	330	175	4.8	5.6	3.0	26.0	78.0	20.0	18	7	15
568.....	450	360	205	7.7	6.1	3.5	19.0	65.0	19.0	41	9	18
852.....	650	380	300	11.0	6.5	5.1	17.5	57.5	19.0	63	11	27

placed upon wax and in almost innumerable points a thin coating is developed as the oil spreads. This may be called the first stage, during which the wetting power of the aqueous phase for wax determines the manner and extent of contact between spray and solid. Displacement of the aqueous phase by oil corresponds in general to that found when a drop of oil and a drop of solution of the accessory substance are placed adjacent to one another upon a waxed slide. As the surface becomes changed from wax to oil, the first stage merges into the second, in which the wetting power of the aqueous phase for a surface of oil determines the degree of contact. Displacement of the aqueous phase by additional oil from the spray is very rapid and complete. Hence the major portion of the primary deposit is laid down during this period.

Secondary deposit is that part of the total oil which reaches the surface from adhering drops or sheets of emulsion when the water in the latter evaporates. The data needed for determination of its magnitude are given in figure 6, *if the assumption is made that the aqueous phase which is left upon the bottles at the conclusion of spraying has the same composition as the original emulsion*. The results are given in table 3.



With low concentrations of the accessory substances, only a small portion of the total oil is contributed by the secondary deposit; but at higher concentrations, this is increasingly important, particularly in the case of blood albumin.

There is evidence from visual observation that the assumption made in these calculations is not valid in all cases; for the residue of emulsion resembles the product formed by creaming, particularly when the larger amounts of accessory substances are present. That is, under the circumstances in which the drops elongate as they roll down the surface, the emulsion becomes enriched and more oil is left as a secondary deposit than is calculated from the weight of emulsion upon the surface. Hence the data of table 3 on secondary deposit should be taken as minimal values.

In general, high secondary deposit is undesirable, for such oil will be left in very localized regions and can contribute to the general deposit only by spreading upon the surface. While this may occur to some extent with oil, it is impossible in the case of a suspended solid such as bordeaux mixture or lead arsenate. Consequently the secondary deposit of such substances is of little value, except with such pests as are attracted to spotty deposits of toxic material—for example, the walnut husk fly, *Rhagoletis completa* Cresson.<sup>6</sup>

The nature of the curves relating total deposit with concentration of accessory substance can be qualitatively explained for blood albumin, hemoglobin, and sodium oleate in terms of the wetting power of the aqueous phase, ease of replacement of the aqueous phase by oil, and stability of the emulsions. The characteristic differences between typical wetting agents and typical emulsifiers are consistent with their relative abilities to deposit oil. The above properties, however, are complex in nature and give no final answer to the problem.

The information gathered from these experiments indicates the possibility of formulating a theory in terms of three fairly simple properties of an oil emulsion:

1. The retreating angle of contact of the aqueous phase upon the chosen solid. This must be determined under dynamic conditions, and at present high-speed photography seems to offer the best approach.

2. Ease of escape of oil from the minute droplets of the emulsion onto the surface. The difficulty in testing this property is separation of it from wetting power of the aqueous phase.

3. Rate of replacement of the aqueous phase by the oil. Theoretically this is involved in equation 2,  $\gamma_{SB} = \gamma_{SA} + \gamma_{AB} \cdot \cos \theta_{AB}$ . Direct measure-

<sup>6</sup> Personal communication from Dr. A. M. Boyce.

ment of  $\theta_{AB}$ , which may be called the angle of replacement, is very difficult under dynamic conditions, and the displacement method of Bartell and Osterhof (1927) and Bartell and Whitney (1932) is complicated in the present case by the solubility of oil in the beeswax.

*Wetting Agents as Spreaders of Oil.*—The question of the rôle of water-soluble wetting agents in promoting the spread of oil has been discussed by certain previous workers (de Ong, Knight, and Chamberlain, 1927; Smith, 1933; Knight and Cleveland, 1934). Smith, who used the term "spreader" in the same sense in which "accessory substance" is used in this report, said: "It appears that one function of a spreader is to cause the oil to spread on surfaces upon which it will not spread otherwise" and "As the water evaporates, the spreader lays down a coating over the surface over which the oil spreads in a uniform film."

The truth of the second statement is substantiated by the experimental observation that oil spreads over a region in which a protein solution has dried on beeswax several times as fast as upon the untreated beeswax. The protein, however, must be dry. Hence the effect can occur with oil emulsions only after the water has evaporated. The evidence from the replacement experiments indicates that water-soluble accessory materials promote wetting by the aqueous phase only. But there are differences in the tendencies of proteins and of soaps to collect in the various interfaces. Thus, sodium oleate has a strong affinity for the oil-water interface and forms very stable emulsions, which wet wax but poorly because the soap has less affinity for the wax-water interface. Blood albumin and hemoglobin promote wetting by water very strongly but emulsify oil poorly. Possibly an accessory substance somewhat soluble in both water and oil would be retained sufficiently by the oil to promote the spread of the oil upon wax during the time of spraying, but this would necessitate the presence in the molecule of three groups having affinities for water, oil, and wax, respectively.

### SUMMARY

The application of a spray is a dynamic process, and hence attempts to relate the deposit of oil obtained from various emulsions should be based upon measurements made under conditions approximating those of use. With a standard surface of beeswax as the solid sprayed and a standard method of spraying, a study has been made of the relations between concentration of blood albumin, hemoglobin, or sodium oleate and the following properties: amount of the aqueous solution and of the accessory substance deposited in the absence of oil; deposit of oil, of aqueous phase, of accessory substance, and of all components when emulsions were used;

ease of wetting of wax by solutions of the accessory substances and by the entire emulsion; replacement of aqueous phase by oil; and stability of the emulsions.

The amount of oil deposit can be explained at least qualitatively as follows: When only oil and water are present, the emulsion wets poorly, and drops roll on the surface with minimum area of contact. Addition of a protein promotes wetting and opportunity for oil to reach the surface. Hence deposit is increased until formation of large sheets of the aqueous phase upon the surface and resistance to displacement of the aqueous phase by oil lead to a decrease in oil deposit with higher concentration of protein. Soap promotes wetting so little that a corresponding increase in oil deposit does not occur. With all three accessory substances, the increase in stability of the emulsions diminishes oil deposit. Water-soluble substances follow the aqueous phase and increase spreading of oil only after the water has evaporated.

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THE USE OF SELENIUM IN SPRAYS FOR THE  
CONTROL OF MITES ON CITRUS  
AND GRAPES

W. M. HOSKINS, A. M. BOYCE, AND J. F. LAMIMAN

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# THE USE OF SELENIUM IN SPRAYS FOR THE CONTROL OF MITES ON CITRUS AND GRAPES<sup>1</sup>

W. M. HOSKINS,<sup>2</sup> A. M. BOYCE,<sup>3</sup> AND J. F. LAMIMAN<sup>4</sup>

## INTRODUCTION

FOR SEVERAL YEARS it has been increasingly obvious that the standard methods used for control of several plant-infesting mites in California are unsatisfactory (Boyce, 1936).<sup>5</sup> For this reason the California Agricultural Experiment Station projects on the citrus red mite, *Paratetranychus citri* McG., and on the Pacific red spider, *Tetranychus pacificus* McG., have been carried on very actively. One feature of such projects is the examination of new materials under both small-scale and practical conditions. In pursuance of this work, a proprietary selenium-containing preparation called Selocide was tested by Lamiman (1933) against the Pacific red spider on grapes. This material had been used successfully by Gnadinger (1933) for control of the common red spider, *Tetranychus telarius* (Linn.), in greenhouses. The first small-scale field trials on both grapes and citrus were of so much promise that a new project<sup>6</sup> was organized in 1934 for the purpose of making a thorough study of the practical utility of Selocide alone and in combination with other substances for the control of mites affecting citrus and grapes.

It is an interesting coincidence that at about the time when the use of selenium for plant protection was suggested, attention was first directed to this element as a harmful ingredient of animal and human foodstuffs. Since the first settlers went into the semiarid Great Plains region of the United States, a livestock ailment of unknown origin has been noticed in many areas of the present states of South Dakota, Nebraska, Kansas, Wyoming, and other neighboring states. The disorder assumed, in general, either an acute or a chronic form, which became known as "blind

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<sup>5</sup> See "Literature Cited" for further data on citations, which are referred to in the text by author and date of publication.

<sup>6</sup> A special committee was appointed by the Dean of the College of Agriculture to conduct the investigation. The members are: W. B. Herms (chairman), D. R. Hoagland, P. L. Kirk, C. D. Leake, and the authors of this report.

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staggers" and "alkali disease," respectively. The first written account seems to have been made by Madison (1860) and concerns an outbreak among cavalry horses at Fort Randall, Territory of Nebraska, the site of which is now included in South Dakota. Madison attributed the trouble to grazing of the affected animals in a certain ravine. Since that time, occasional references to the disease have been made in agricultural experiment station reports, but little advance was made in determining the cause. Among practical stockmen, it was attributed usually to alkaline drinking water.

In 1929 Franke of the South Dakota Agricultural Experiment Station began the first comprehensive investigation of alkali disease and soon proved (Franke, Rice, *et al.*, 1934) that it invariably followed the ingestion of grains from certain districts in the state. The discovery by Robinson (1933) that selenium was present to the extent of 10–12 p.p.m. in a sample of the so-called "lethal wheat" at once gave a new direction to the investigation and led to extensive feeding trials with both inorganic selenium compounds and seleniferous plants in many laboratories. A warning against hasty adoption of selenium-containing materials as sprays was issued by Nelson, Hurd-Karrer, and Robinson (1933).

In the light of these conditions, the investigation of the California Agricultural Experiment Station was designed to include, in addition to a study of the effectiveness of the selenium preparation for control of mites, determination of the amounts of selenium residue upon fruit as the result of various spray programs, of the accumulation of selenium in the soil beneath sprayed plants, and of the absorption of this selenium from the soil. Also, preliminary plant-rearing experiments and feeding trials of inorganic selenium and seleniferous plant materials were made.

This report includes accounts of the experimental procedures and results, as well as a summary of published information on the general occurrence and toxicity of selenium and a discussion of its probable health hazard when used in the recommended manner upon citrus and grapes.

## CHEMICAL PROPERTIES OF SELENIUM AND ITS COMPOUNDS

Selenium (Se) has an atomic weight of approximately 79 and lies between sulfur and tellurium in the sixth group of the periodic table of the elements. Its closest neighbors in the adjacent groups are arsenic and bromine. In the uncombined state, selenium occurs in several allotropic forms, of which the more common are the grayish-black crystalline, the black amorphous, and the red amorphous. The latter is formed when selenium is reduced from one of its compounds, is the most reactive form

of elementary selenium, and under many conditions is converted to the black crystalline state. The chemical properties of selenium are similar to those of sulfur. Both vary in valence from  $-2$  to  $+6$ . Typical compounds representing the various valences are:  $-2$ , hydrogen selenide,  $\text{H}_2\text{Se}$ ;  $+2$ , selenium sulfide,  $\text{SeS}$ ;  $+4$ , selenium dioxide,  $\text{SeO}_2$ , which unites with water to form selenious acid,  $\text{H}_2\text{SeO}_3$ ;  $+6$ , selenium trioxide,  $\text{SeO}_3$ , which is known only in the hydrated form as selenic acid,  $\text{H}_2\text{SeO}_4$ . The two oxides and their corresponding acids and salts are strikingly different in behavior: the tetravalent state is readily formed when nearly any ordinary oxidizing agent acts upon free selenium or its lower compounds, but only the most powerful oxidizing agents—for example, permanganate—can raise it to the hexavalent state. However, when fully oxidized, selenium can be reduced only with difficulty. Hence the selenate compounds are usually comparatively inert. On the contrary, the selenite state is reactive and may be readily reduced to a lower valence.

Despite its close chemical relationship to sulfur, selenium until recently aroused little interest as a material for plant protection. The work of Lougee and Hopkins (1925) proved the toxicity of several selenium compounds to certain fungi, but damage to sprayed trees was severe.

A 30 per cent solution of a mixture of potassium hydroxide, ammonium hydroxide, sulfur, and selenium in the proportions corresponding to the empirical formula  $(\text{K NH}_4 \text{ S})_5 \text{ Se}$  is the proprietary product called "Selocide." It contains 48 grams of selenium per liter, or approximately 6.4 ounces of selenium per gallon. Dilutions of 1:100 and 1:1,000 give sprays containing 0.0480 and 0.0048 per cent selenium respectively, and other dilutions are of proportional concentrations. The first work done for control of the common red spider was usually at a dilution of 1:200 (Gnadinger, 1933) but Lamiman (1933) found much more dilute solutions—for example, 1:600 and 1:800—to be effective outdoors against the Pacific red spider on grapes, and these same concentrations have been used widely on citrus. For proper wetting of most leaf and fruit surfaces, a wetting agent, such as a soluble protein, is advisable.

The reactions which occur when a concentrated solution of Selocide is diluted with water, as in preparing a 1:800 spray mixture, do not appear to be known. Observation shows, however, that a large part, if not all, of the selenium is liberated as very finely divided, dark-red particles, which appear to be the red allotropic form of the element. When finely ground sulfur is added to Selocide, the residue has a persistent effect which is shown by neither component alone (see p. 119). There is no chemical evidence that a compound is formed, but the effect may possibly be due to some new product, such as selenium sulfide.

## SELOCIDE ON CITRUS AND WALNUTS

*The Citrus Red Mite.*—The proprietary selenium preparation Selocide has been rather extensively studied in connection with the control of the citrus red mite (spider), *Paratetranychus citri* McG., in southern California during the period from 1933 to 1937. This mite is one of the major pests of citrus in many areas (Boyce, 1936). The total acreage of citrus affected probably exceeds 125,000 acres.

Until the advent in about 1925 of the highly refined petroleum oils for citrus sprays, sulfur in some form had been largely used for the control of the citrus red mite. During the past ten years, however, the use of sulfur or its compounds for the control of this mite has been very limited. This fact is due principally to the following causes: (1) superiority of oils for this purpose and the more general use of oils as a combination treatment for control of scale insects and this mite; (2) the unsatisfactory results in the control of this mite by sulfur as compared with the results secured in former years; and (3) hazard of fruit injury caused either by dry, hot winds in certain sections or by a sudden increase in daily temperature after sulfur applications. Thus, at present, petroleum oil is the principal material used for the control of the citrus red mite.

In many sections, under ordinary conditions, one application of oil spray in the summer or fall affords satisfactory protection from injury by this mite for about one year. However, after the use of an oil spray, if climatic conditions are extremely favorable to mite development throughout the winter and spring or throughout the summer, there usually develops a need for mite control with a material that contains little or no petroleum oil. In the coastal areas, there is commonly a necessity for citrus-red-mite control in the spring or early summer, even though oil spray was applied the preceding summer or fall. From the standpoint of adverse tree reaction, more than one treatment of oil spray at full dosage on citrus during a year is generally considered inadvisable. A large number of growers prefer to abstain from the use of oil altogether and therefore employ HCN fumigation in the control of scale insects. Fumigation usually has no appreciable effect in citrus-red-mite control; in fact, the mite populations commonly increase more rapidly after fumigation than otherwise, since many of their natural enemies are killed. Evidently there is an urgent demand for a material other than oil for use in the control of the citrus red mite.

Many laboratory tests and several hundred control experiments in the field were conducted in the various citrus-red-mite areas. These were designed principally to determine the most effective (1) concentration of



Selocide; (2) adjuvant, such as lime-sulfur, wettable sulfur, or oil; and (3) spreading and wetting agent. The data show that Selocide at 1:800 is as effective as any of the higher concentrations that are practical to use. An adjuvant is essential for maximum efficiency, the most satisfactory ones apparently being lime-sulfur at 1:300, wettable sulfur at 1 to 2 pounds per 100 gallons of spray mixture, light-medium mineral oil at 1:300, and a combination of the oil and wettable sulfur.

The Selocide-and-lime-sulfur or wettable-sulfur combination affords a high degree of kill of the mites contacted. It kills only a small percentage of the eggs; but a residuum is deposited on the tree that kills the young mites shortly after they are hatched, even during relatively low temperatures and under conditions when the residuum from a 2 per cent lime-sulfur spray or regular sulfur dust is ineffective. Definite information regarding the chemical nature of this residuum is not available. The use of either of these Selocide-sulfur combinations may not effect satisfactory control when rains follow the application within a period of several weeks and wash the residuum off the foliage and fruit, for under these conditions the mites that subsequently hatch survive.

The Selocide-oil combination effects a high initial kill of the mites and eggs contacted; however, there is apparently no residual effect from the use of this combination, and, therefore, unless the application is very thorough, the results may not be satisfactory. A comparison of many parallel field experiments with the Selocide-lime-sulfur and Selocide-wettable-sulfur combinations versus the Selocide-oil combination shows that the last-named has generally afforded a higher degree of control, although in certain experiments the two sulfur combinations were superior.

When it became evident that each combination possessed certain specific merits, tests were conducted in which both lime-sulfur and oil, each at 1:300, and both wettable sulfur at 1 pound per 100 gallons and oil at 1:300, were combined with Selocide. Tree injury occurred under certain conditions with the Selocide-lime-sulfur-oil combination. However, the Selocide-wettable-sulfur-oil combination appears to be noninjurious to the tree and has afforded very effective control of the mite. A fairly large acreage has been treated commercially with this combination with satisfactory results.

Several spreading and wetting agents, such as sodium caseinate, calcium caseinate, blood albumin, soaps, sulfonated plant oils, and sulfated higher alcohols have been used with Selocide in the several combinations. According to present information, sodium caseinate is the most practical spreading and wetting agent for use with Selocide. Blood albumin should

not be used on citrus in any Selocide combination in which oil is included, since extensive tests have shown that serious staining or spotting of the fruit may occur from this combination of materials.

In summing up the field studies with Selocide on citrus, it may be said that this material, used according to the following formula, has given satisfactory control of the citrus red mite and is the one suggested for use where Selocide is considered:

Selocide.....	1 pint
Wettable sulfur.....	1 pound
Light-medium oil <sup>7</sup> .....	$\frac{1}{2}$ gallon
Sodium caseinate.....	3 to 4 ounces
Water.....	100 gallons

The regular dusting sulfur may be used in the same amount and rendered wettable by mixing the caseinate with the dry sulfur in a large bucket and then adding water under pressure with the spray gun until the sulfur is suspended in the water.

With regard to the choice of oil, the straight oil, as used in tank-mix, appears to have certain advantages over paste emulsions or emulsive oils, since both of the latter types of oils contain wetting and spreading agents which, when included with the caseinate regularly used with Selocide, may possibly cause excessive runoff of the spray mixture. In commercial practice, however, the paste emulsions and emulsive oils are used with success.

The order in which the several materials are put into the spray tank is of importance. The sulfur-casein, prepared as previously indicated, should be put into the tank first, when the water is about level with the agitator shaft. The Selocide should be added next when the tank is about one-third filled, and the oil added last when the tank is about two-thirds filled. If paste emulsion or emulsive oil is used, the recommendations of the manufacturer should be followed with regard to when the oil is to be added; however, in all instances the sulfur should be put into the tank before the Selocide.

*Other Mites and Insects on Citrus.*—The Selocide-sulfur-oil combination has afforded satisfactory control of the six-spotted mite, *Tetranychus sexmaculatus* Riley, on citrus. It has not been entirely satisfactory, however, in the control of the silver or rust mite, *Phyllocoptes oleivorus* (Ashm.), which is of importance only sporadically in limited areas in San Diego County. Since this mite is readily and inexpensively controlled through applications of sulfur applied as a dust, Selocide would not be considered in this connection unless the citrus red mite or the six-

<sup>7</sup> Unsubstantiated residue 92 per cent; distillation range: 10 per cent at 587° F, 50 per cent at 632° F, 90 per cent at 702° F.

spotted mite or both species were also present. Under such conditions, the silver mite could probably be satisfactorily controlled by increasing the amount of wettable sulfur from 1 pound to 4 or 6 pounds per 100 gallons in the Selocide-sulfur-oil combination.

Selocide as used, either experimentally or commercially, has not been shown to possess measurable value in the control of any of the *insect* pests of citrus with the possible exception of the citrus thrips, *Scirtothrips citri* (Moult.). In this particular instance, those thrips that are contacted by the Selocide spray mixture are killed; and when either lime-sulfur or wettable sulfur is included in the spray mixture, the residuum is effective in destroying the newly hatched nymphs for a period of about 1 week following the treatment. The Selocide-sulfur-oil combination would probably be of considerable value for the combined treatment of citrus red mite and the citrus thrips under certain conditions if the amount of wettable sulfur were increased from 1 pound to 4 or 6 pounds per 100 gallons, as recommended for the silver mite.

*Compatibility of Selocide with Other Materials Commonly Used in Sprays on Citrus.*—Selocide may be used with nicotine sulfate for the combined control of the citrus aphids, *Aphis spiraecola* Patch, *Aphis gossypii* Glover, and *Toxoptera aurantii* (B. d. Fonse) and several species of mites previously mentioned.

Where zinc compounds are used for the correction of mottle-leaf or little-leaf (Parker, 1938) of citrus trees, either zinc oxide or zinc sulfide may be satisfactorily combined with Selocide in the spray mixture. However, the zinc-sulfate-hydrated-lime mixture is incompatible with Selocide.

*Limitations to the Use of Selocide on Citrus.*—The use of Selocide on Valencia oranges in the coastal sections of Los Angeles County and the northern fringe of Orange County is attended with considerable danger of injury to the fruit during the period from late winter until the mature fruits are harvested. In certain seasons in this particular area, a rind weakness develops which is apparently due to environmental conditions. When sulfur is applied, it commonly accentuates this condition, with the result that one or more spots may develop on a fruit. Affected fruits either drop before harvest or otherwise are marketed in the lower grades. The sulfur or other constituents of Selocide contribute to the incidence of this type of fruit spot when other environmental conditions are favorable.

In the Chula Vista area of San Diego County, the peel of lemons not uncommonly becomes stained in small areas, or spotted, after use of Selocide. Affected fruits do not color uniformly; and while their quality is

apparently not impaired otherwise, they are not marketable in the higher grades for this reason. In this particular area, the cause of the stain on the peel of the fruit appears to be related to the occurrence of relatively large amounts of certain salts commonly present in the water that is used for spraying.

A type of stain on the peel of Navel oranges similar to that described above on lemons occasionally results from the application of Selocide. In the instances observed, however, the treatment was applied at the season when the fruit was "breaking color." Through further studies, the causes of the stain on the peel of lemons and oranges may perhaps be determined, and the difficulty may be prevented.

When excessively high temperature conditions prevail within about 10 days after Selocide treatment, some of the fruit that is exposed to the direct sunlight is commonly injured. The injury is typical of that due to high temperatures after the application of sulfur.

*Effect of Selocide on the General Vigor and Productiveness of Citrus Trees.*—Thousands of acres of oranges and lemons have been treated with Selocide during the past five years at different seasons of the year and under widely varying conditions. A relatively large acreage has received as many as five treatments and a smaller acreage as many as seven or more treatments during that period. In no instance has there been any observable adverse effect upon the general vigor or productiveness of such treated trees, nor has there been observed any impairment in the texture or quality of the fruit other than the features previously mentioned.

*Studies with Selocide on Persian Walnuts.*—Selocide has been fairly extensively used experimentally and commercially on Persian walnuts in the control of the common red spider. One treatment annually of the material used according to the formula previously given for citrus has afforded entirely satisfactory control. While treatment for this mite is necessarily applied in midsummer in the hot interior valley areas, no evidence of injury due to the material has been observed.

*Effect of Selocide on Spraymen.*—Many spraymen have applied large volumes of Selocide spray mixture to citrus over relatively long continuous periods during several years without any observed deleterious effects to date upon their health and with no more personal discomfort than would be experienced through the application of light dosages of lime-sulfur spray. More than 40,000,000 gallons of Selocide spray have been applied in the United States, much of it indoors in greenhouses. Gnadinger (1937) is authority for the statement: "No workman engaged in the manufacture or packing of Selocide has experienced any illness or injury traceable to Selocide or remotely resembling selenium poisoning."



## SELOCIDE AS A CONTROL FOR THE PACIFIC RED SPIDER ON GRAPES

The Pacific red spider, *Tetranychus pacificus* McG., which has become a serious pest of grapes in the grape-growing areas of northern California, was first observed as a grape pest in 1928 in the San Joaquin Valley.

The habits of this mite are such that a satisfactory control is very difficult. In late summer and early fall, the adult females hibernate beneath the bark of the grapevines, where they may be found in groups of a few to several hundred. As many as 2,000 have been taken from the bark of a single vine, with a survival of 50 to 80 per cent the following spring. These overwintering females emerge in March and feed on the opening buds or may be found on various weeds, such as filaree, sheperd's-purse, and malva. The appearance of the mites is influenced by weather conditions and during a cool spring may be delayed for two to three weeks; thus the maximum infestation may be delayed until late summer.

Each female deposits from 50 to 100 eggs over a period of 2 weeks to a month, and only 10 to 14 days are required for hatching and development of the mite under summer conditions, with the result that several generations may be produced in a single season. The greatest populations occur in late July or August, when infestations ranging from 600 to 1,400 mites per leaf have been observed on leaves showing severe mite injury. The rapidity with which these mites may multiply is indicated by the increase in the average number of Pacific red spider per leaf from 18.8 on June 12 to 343.7 on August 12, with a maximum of 1,458 on untreated vines; and from 0.2 on June 12 to 192.1 on August 12, with a maximum of 296 on treated vines. A large population of mites in combination with high temperatures and low humidity then produces severe vine injury by defoliation and reduces the quality of the crop by exposure to sunburn as well as by lowered sugar content.

Early attempts to control this mite by means of the accepted methods of red-spider control resulted in a number of problems. Until about 1925, when highly refined mineral-oil sprays were developed for summer use, application of sulfur, in its various forms, was the recognized red-spider control. Sulfur dusts are ineffective in controlling the Pacific red spider, even though several applications are made in the same season. Lime-sulfur and sulfur sprays, the chief control before the advent of summer oil sprays, were unsatisfactory due to the excessive injury to new growth in early summer and to the blotchy residue left on the fruit by later applications. Summer oil sprays, the method most widely used against red spiders on deciduous trees since 1925, gave excellent initial results

but did not prevent reinfestation by the few individuals escaping the treatment. These sprays also removed the bloom from the grapes and produced shiny, soft, flabby berries, and thus reduced the quality and market value of the product. However, oil sprays can be used to reduce the infestation of new growth by the overwintering females before the blossoms appear.

Selocide,<sup>a</sup> a spray material developed by Gnadinger (1933), while conducting experiments on the control of the common red spider, *Tetranychus telarius*, in greenhouses, was used in 1932 (Lamiman, 1933) under field conditions. In these experiments, the Selocide concentrate, at a dilution of 1:800 (1 gallon to 800 gallons of water), with a cocoanut-oil soap spreader, was highly effective under field conditions, whereas a dilution of 1:200 had previously been recommended for greenhouse conditions. As these applications were made in July, the 95–100 per cent control obtained on juice-grape varieties prevented serious reinfestation.

In 1933 the experimental work was extended to include the Thompson Seedless variety, and applications were begun as soon as mite damage appeared in early June. Reinfestation of the vines became apparent after about 4 weeks, even though the initial kill was high; in many cases additional applications were needed to prevent defoliation.

During the same year, Gnadinger (1933) found that the effectiveness of the Selocide spray in the control of the citrus red mite was greatly increased by the addition of lime-sulfur (1:300) or a light medium mineral oil (1:300). Since neither of these combinations could be applied on grapes, wettable sulfur<sup>o</sup> in varying amounts was added to the Selocide spray in 1934 and 1935. Reinfestation was greatly delayed, and where applications were made in late June or early July, the vines retained their foliage and matured the crop, although some mite damage occurred before the mites began to hibernate in August and September.

The effectiveness of the various concentrations of Selocide without sulfur and of other materials is shown in table 1. While a dilution of 1:800 gave satisfactory results under certain conditions, a 1:600 or 1:500 dilution was more effective, especially on the Thompson Seedless variety with its denser foliage. Table 2 shows the results obtained by the addition of varying amounts of sulfur to the different dilutions of the Selocide spray. At the dilutions used, Selocide did not produce any injury to vines or fruit.

The results shown in the tables were obtained by collecting leaves at

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<sup>a</sup> The effect of Selocide on spraymen is discussed in the previous section, p. 122.

<sup>o</sup> The wettable sulfur was prepared by adding either blood-albumin spreader or caseinate spreader to ordinary fine dusting sulfur; the combination was then mixed into a paste and added to the spray tank.

random in the treated areas; from each group, 20 leaves were selected at random. On these leaves, five areas (each 5 sq. cm) were marked off with a circular rubber stamp. These areas were arbitrarily selected as being those most likely to be infested by the mites. Comparisons of the count areas with the remainder of the leaf showed that approximately one-

TABLE 1

COMPARISON BETWEEN SELOCIDE WITHOUT SULFUR AND OTHER MATERIALS  
FOR THE CONTROL OF PACIFIC RED SPIDER ON GRAPES

Materials used	Date of application	Average population per leaf based on number in count areas					
		Before treatment	After 3 days	After 10 days	After 30 days	After 50 days	After 70 days
Selocide 1:400+4 oz. blood-albumin spreader* to 100 gals.....	{ June 23	31	2	1	3	39	..
	{ July 7	37	2	6	21	53	..
Selocide 1:400+8 oz. Ortho casein and 4 oz. blood-albumin spreader to 100 gals.....	July 30	66	32	..	91	..	..
Selocide 1:600+4 oz. blood-albumin spreader to 100 gals.....	June 2	69	13	3	4	20	90
Selocide 1:600+4 oz. blood-albumin spreader to 100 gals.....	July 7	40	4	7	33	103	..
Selocide 1:600+12 oz. Fluxit† to 100 gals.....	June 2	69	6	2	0.7	14	57
Selocide 1:600+8 oz. blood-albumin spreader to 100 gals.....	June 20	42	2	3	31	..	..
Selocide 1:600+8 oz. Ortho casein and 4 oz. blood-albumin spreader to 100 gals.....	July 26	91	11	41	86	..	..
Light-medium tank-mix oil 1½ per cent.....	June 2	69	1	4	4	30	..
Wettable sulfur 6 lbs. to 100 gals.....	{ June 17	43	6	7	55	..	..
	{ June 17	38	11	15	55	..	..
Wettable sulfur 6½ lbs. to 100 gals.....	July 8	37	13	19	47	78	..
Lethane‡ 420, 1:600.....	June 19	45	17	30	64	..	..
Lethane 420, 1:300.....	July 30	27	26	35	..	..	..
Ortho derris 4 lbs. to 100 gals.....	June 19	36	7	20	87	..	..
Untreated.....	{ June 5†	12	..	33	48	98	..
	{ June 18†	32	..	54	71	..	..

\* A commercial product containing 1 part powdered blood albumin (about 98 per cent water-soluble) and 3 parts fuller's earth.

† A commercial product consisting chiefly of calcium caseinate plus excess lime.

‡ First counts made.

§ A commercial organic thiocyanate.

third of all the mites present occurred in the count areas, which represented an average of 20 per cent of the leaf surface. This method made possible the examination of a greater number of leaves from each experimental area.

The results of the tests described above may be summarized as follows: The Selocide-wettable-sulfur combination not only affords a high initial kill of the mites contacted, but it also has a lasting effect, for a residuum is apparently deposited which kills the young mites after they have

hatched out and the mites which were in the molting stage at the time of the application. At present, the only satisfactory summer control for the Pacific red spider on grapes is the use of this Selocide-wettable-sulfur combination. However, oil sprays may be used in spring and early sum-

TABLE 2  
EFFECT OF ADDING VARYING AMOUNTS OF SULFUR TO SELOCIDE IN THE CONTROL OF THE PACIFIC RED SPIDER ON GRAPES

Materials used	Date of application	Average population per leaf based on number in count areas					
		Before treatment	After 3 days	After 10 days	After 30 days	After 50 days	After 70 days
Selocide 1:400+6½ lbs. sulfur and 12 oz. Fluxit† to 100 gals.....	July 8	37	0.0	0.0	0.2	0.8	..
Selocide 1:400+2 lbs. sulfur and 6 oz. blood-albumin spreader* to 100 gals.....	July 30	57	0.1	....	0.1	....	..
Selocide 1:500+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.....	July 2	34	2	0.2	9	24	..
Selocide 1:500+2 lbs. sulfur and 6 oz. blood-albumin spreader to 100 gals.....	July 14	58	0.6	0.1	3	..	..
	July 31	31	0.3	1.0	2	..	..
Selocide 1:600+2½ lbs. sulfur, 8 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.....	July 24	33	0.1	....	3	..	..
Selocide 1:600+6½ lbs. sulfur and 8 oz. Fluxit to 100 gals.....	July 11	42	0.2	0.9	2	21	..
Selocide 1:600+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.....	June 3	14	1.0	1.0	1.0	29	..
	June 20	18	0.1	1.0	1.0	41	85
Selocide 1:600+1 lb. sulfur and 8 oz. blood-albumin spreader to 100 gals.....	June 3	11	0.4	2	9	54	..
Selocide 1:600+½ lb. sulfur and 8 oz. blood-albumin spreader to 100 gals.....	June 3	13	1.0	2	28	64	..
Selocide 1:800+6½ lbs. sulfur and 8 oz. Fluxit to 100 gals.....	July 11	50	....	1.0	2	18	..
Selocide 1:800+5 lbs. sulfur, 4 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.....	July 12	54	1.0	3	4	45	..
Selocide 1:800+2 lbs. sulfur and 8 oz. blood-albumin spreader to 100 gals.....	July 18	13	1.0	1.0	1.0	3	29
Selocide 1:800+1 lb. sulfur and 6 oz. blood-albumin spreader to 100 gals.....	July 28	33	0.3	2	3	9	25
Selocide 1:1,000+5 lbs. sulfur, 4 oz. Fluxit, and 4 oz. blood-albumin spreader to 100 gals.....	July 12	68	2	4	26	63	..

\* A commercial product containing 1 part powdered blood albumin (about 98 per cent water-soluble) and 3 parts fuller's earth.

† A commercial product consisting chiefly of calcium caseinate plus excess lime.

mer against the overwintering adults on the new growth before the grapes develop.

## SELECTION OF SAMPLES AND METHODS OF ANALYSIS

*Collection of Samples.*—Fruit and leaves were selected at random, with special care to disturb the surface as little as possible. They were placed in clean bags, packed in boxes, and taken or sent to the laboratory at once.



Selenium adheres well and it is felt that no serious losses occurred during the necessary handling. Four to eight fruits were used for each analysis. Grape samples were composed of portions of several bunches from different vines whenever possible.

Soil samples were taken by members of the Station staff at Riverside. In order to obtain data for extreme conditions, the samples were taken near the edge of the trees where drip of spray would be at a maximum. Separate samples were taken in each case at the depths of 0-6 inches, 6-12 inches, 12-24 inches, and 24-36 inches. In most cases these were analyzed separately.

*Review of Methods of Selenium Analysis.*—Many methods have been devised for the quantitative analysis for selenium. Those which appear to have been most successful are based upon the following properties of the element: (a)  $\text{Se}^{+6}$  boiled with 3 to 12 *N* HCl is reduced to  $\text{Se}^{+4}$  (Norris and Fay, 1896, Noyes and Bray, 1907); (b)  $\text{Se}^{+4}$  in the same hot acid solution is reduced to free selenium by  $\text{SO}_2$ , hydrazine, hydroxylamine, etc.; (c) metallic selenium reacts with strong nitric acid to give only selenious acid (Lyons and Shinn, 1902); (d) selenious acid reacts with iodide to liberate free iodine, which can be estimated with standard thiosulfate solution. For large amounts, the weighing of free selenium liberated as in *b*, is undoubtedly subject to least objection, but gravimetric methods are not possible with the amounts found in a reasonable sample of most plants or soils. The method used for many analyses in the United States Department of Agriculture Bureau of Chemistry and Soils (Williams, 1937) depends upon the color developed when free selenium is liberated under such conditions that it forms a comparatively stable suspension. In the present work, fair success was had with this method, but the tendency of the suspended material to assume any color from pink through brown to black made comparison with a standard very uncertain. Addition of gum arabic (Association of Official Agricultural Chemists, 1935) made no decided improvement. Other workers have had the same kind of difficulties (Mathews, Curl, and Osborn, 1937).

Preliminary experiments indicated that the reaction,  $\text{H}_2\text{SeO}_3 + 4\text{K}^+ + 4\text{I}^- + 4\text{H}^+ \rightarrow \text{Se} + 2\text{I}_2 + 3\text{H}_2\text{O} + 4\text{K}^+$ , with consequent titration of the free iodine with standard thiosulfate, is suitable for the iodometric determination of small amounts of selenium. This method was criticized by Gooch and Reynolds (1895), who were unable to get complete reduction in the cold. However, Norton (1899) showed that a large excess of iodide in approximately 0.5 *N* hydrochloric acid gave excellent results except with large amounts of selenium. Recently, Berg and Teitelbaum (1928) raised two more objections: (1) the liberated iodine is absorbed by the colloidal

selenium and does not react quantitatively with thiosulfate, and (2) the end point is obscured by the color of the colloidal selenium. These complications are serious when selenium is present in amounts of 500 $\gamma$  (0.5 mg) or over; but with 200 $\gamma$  (0.2 mg) of selenium or less, no serious difficulties have been encountered in several hundred titrations, though the end point is decidedly different from that of an ordinary iodometric titration.

*Tests of Analytical Methods with Pure Selenium Compounds.*—Selenium dioxide was prepared by treating black selenium with concentrated nitric acid, warming gently to remove most of the excess acid, and then cautiously subliming onto the bottom of a cold porcelain evaporating dish. The first crop of crystals was treated with a drop of nitric acid and resublimed. The final product consisted of snow-white needles which were somewhat hygroscopic. A stock 0.1 *M* solution of selenious acid was prepared gravimetrically and diluted to 0.010 or 0.001 *M* as required. The reagents used were all of C.P. grade.

The purity of the contents of a stock bottle of sodium thiosulfate crystals was determined by numerous titrations against a standardized dichromate solution. The results were almost theoretical. Hence, for routine work, 0.05 *M* thiosulfate solutions were made at frequent intervals by weighing out the crystals, adding to freshly distilled water, and thereafter protecting from light and air. Each morning a fresh 0.001 *M* thiosulfate solution was prepared by dilution.

The usual precautions were taken to ensure that the potassium iodide did not liberate free iodine when put into solution. From a bottle of satisfactory crystals, a small volume of 25 per cent solution was made each morning. A small pinch of sodium bicarbonate was used to stabilize the solution, which also was protected from strong light.

The acid needed in the reaction proved to be the principal source of trouble. With high-grade hydrochloric or sulfuric acid at approximately 1 *N* concentration, no difficulty was encountered in securing zero blanks or stoichiometric reactions for a wide variation in the amount of selenium. However, the reaction had to proceed in the presence of nitric acid because that compound is the most convenient for oxidizing free selenium (formed as in step *b*, p. 127) to the tetravalent form. Two separate considerations are involved: (1) the strength of acid needed to enable the reaction between selenite and iodide to proceed at a satisfactory rate, and (2) the strength of nitric acid which can be tolerated by the excess iodide ion. These had to be studied together, for it was possible that the colloidal selenium produced in the first reaction had an effect upon the second one. Complete expulsion of the excess nitric acid by repeated

evaporation to dryness on a water bath, as suggested by Beath, Eppson, and Gilbert (1935), was tried; but in some cases loss of selenium occurred.

It is well known that the lower oxides of nitrogen react with iodide ion. Hence, care must be taken to ensure their absence. For micro-iodometric analysis, the color of nitric acid is not a safe guide. It has been found that vigorous aeration for 1 hour of any high-grade nitric acid except very brown lots suffices to remove the offending compounds. The distilled water used must be of high quality. A blank containing nitric acid, iodide, and starch should be tried each day in order to check the condition of the acid and of the water.

The most suitable amount of nitric acid for the iodometric determination of selenite was determined by adding 1.000 cc of 0.001 *M* selenious acid solution (79.2 $\gamma$ Se) to the indicated volumes of concentrated nitric acid in a small Erlenmeyer flask and adjusting the volume to 10 cc; 0.25 cc of 25 per cent potassium iodide solution was added; and the flask was stoppered and allowed to stand for 5 minutes. The liberated iodine was then titrated with standard 0.001 *M* sodium thiosulfate solution. Starch solution was used in the ordinary way. After titration, the solutions were set aside and retitrated 10 minutes after introduction of the iodide. The titrations of the solutions containing the different volumes of nitric acid were as follows:

Nitric acid, in cc	Thiosulfate solution, in cc	
	5 minutes	10 minutes
0.05.....	0.600	0.740
0.10.....	0.680	0.950
0.20.....	2.447	2.780
0.40.....	3.700	3.850
0.60.....	4.020	4.078
0.80.....	4.090	4.175
1.00.....	4.191	4.222
1.40.....	4.232	4.312
1.60.....	4.380	4.445

The results show that 0.60 to 0.80 cc nitric acid give satisfactory results for the titration at 5 minutes and no serious error after 10 minutes. The theoretical volume of thiosulfate solution is 4.000 cc.

Since concentrated nitric acid is approximately 15 *N*, the desired concentration in the reaction vessel (0.60 to 0.80 cc in 10 cc) is about 0.9 to 1.2 *N*. Concentrations of nitric acid much higher than this slowly liberate free iodine from the excess iodide, as is shown by the data above.

The accuracy to be expected from the direct titration of varying

amounts of selenium was determined by titrating known amounts of selenious acid solution. The average results were :

Se added, $\gamma$	Se found, $\gamma$
4	5
7.9	8.4
15.8	16.9
31.6	33.8
47.5	49.8
63.3	66.1
79.2	82.0
158.4	161
237.6	241

With the proper conditions ascertained for the iodometric determination of soluble tetravalent selenium, the next step was to study the conditions necessary for the quantitative reduction of selenite, separation of the free element, and its reoxidation to the tetravalent state. Noyes and Bray (1927, p. 323) suggested the reduction of  $\text{Se}^{+4}$  by hydroxylamine-hydrochloride in approximately 5 *N* hydrobromic acid solution at steam-bath temperature. Preliminary experiments with selenious acid showed the same conditions to be satisfactory when hydrochloric acid was substituted for the hydrobromic. The characteristic brick-red precipitate is obtained except with very small amounts of selenium or when interfering substances are present. However, the change to the compact black form may occur very rapidly, so that the color is not a sure indication of the true conditions. The procedure adopted after many tests was : The solution, held in a 25  $\times$  150 mm test tube, is made 4–6 *N* with hydrochloric acid in a total volume of 15–20 cc. It is placed in a boiling water bath. When it is hot, 2 cc hydroxylamine hydrochloride solution are added. Heating is continued for 1 hour with the addition of 1 cc more of the hydroxylamine solution. Centrifuging is done either at once or later. Addition of a very little finely divided asbestos aids in collecting the precipitate. If the asbestos is a thoroughly washed high-quality product, it does not affect the subsequent titration.

For the separation of the selenium from the liquid, two types of stick filter have been used with equal satisfaction. The first consisted of a capillary tube, the bore of which was slightly enlarged at the lower end. This swelling was filled with finely divided asbestos pulp. When connected with a suction, the asbestos became very tightly packed but still allowed liquid to pass with reasonable speed, 0.5 to 1.0 cc a minute. The other filter had a larger opening at one end which was filled with finely ground glass sintered in place. When a very thin pad of asbestos was pulled over this by suction, rapid filtering was possible without loss of

selenium. During the process of filtering, the inside of the test tube should be washed down several times with water, so that eventually all soluble materials are removed and the selenium is left with practically no water.

To oxidize the selenium, 0.8 cc of concentrated nitric acid is carefully run down the inside of the test tube and the outside of the stick filter. The tube is then placed in a steam bath for about 5 minutes and is turned about until the hot acid has had an opportunity to dissolve all the selenium present. The filter stick is put into a 125-cc Erlenmeyer flask and washed inside and out with water. It is then laid aside, and the contents of the test tube are washed quantitatively into the flask. With the total volume of liquid about 10 cc, the flask is placed on a sand bath and heated just to boiling. Spattering must be avoided. Vapors are removed by blowing out the flask two or three times with the breath. The flask is then cooled in running tap water, the contents adjusted to approximately 10 cc, and a stream of nitrogen or carbon dioxide is bubbled through for 5 minutes; 0.25 cc of 25 per cent potassium iodide solution is next added, and the stoppered flask is allowed to stand for 5 minutes. Titration is then made with 0.001 *M* sodium thiosulfate solution and 1 per cent starch solution.

The use of a very little asbestos to aid in collecting the selenium and in filtering out the liquid does not interfere with the titration; larger amounts obscure the end point. The color change involved in the titration is somewhat different from that of an ordinary iodometric determination, for the liberated selenium gives a golden color to the solution which is largely obscured by the iodine but becomes evident just at the end point. The reason for heating the solution to boiling, blowing out vapors, and flushing with an inert gas is chiefly to remove the reduction products of nitric acid, which react with iodide ion. Replacement of the air in and above the liquid is advisable also because the liberation of iodine by oxygen is thereby minimized. When the above process is carried out carefully, the amount of nitric acid finally left is approximately 0.6 cc, which, as shown earlier, is suitable for the reduction of selenite and titration of the liberated iodine.

The results of many experiments with different amounts of selenium carried through the processes of precipitation, reoxidation, and titration are summarized as follows:

Se added, $\gamma$	Se found, $\gamma$	
	Range	Average
19.8.....	19.0-20.0	19.5
29.6.....	38.2-41.8	39.5
79.2.....	70.6-78.5	76.7
158.4.....	152-157	154.6



Recovery was satisfactory over the range studied, which covered nearly all of the subsequent analyses on fruit and soils.

*Analysis for Selenium in Plant Material.*—Before the accuracy of recovery of selenium added to fruit could be studied, it was necessary to decide upon a method for destroying the organic matter contained in the skin and pulp of the fruits concerned without loss of selenium. The numerous methods which have been described for wet (Robinson *et al.*, 1934; Williams and Lakin, 1935) or dry (Mathews, Curl, and Osborn, 1937) combustion of plant material are time-consuming and are not without objection when selenium is to be determined quantitatively, for the free element and several of its compounds are readily volatilized. In an effort to prevent such loss, attention was directed to the simplified Karns (1932) apparatus devised by von Kolnitz and Remington (1933). In this, within a closed system, a cartridge of the dried vegetable material is thrust slowly into an oxygen flame and the products of combustion are absorbed in a train of wash bottles. This proved so successful that most of the materials reported on below were burned by that procedure. Two  $1\frac{1}{2} \times 8$  inch washing tubes containing about 4 inches of distilled water above coarse Pyrex sintered glass filters fused in near the bottom were used to catch the products of combustion. Tests proved that most of the selenium was trapped in the first tube and that none escaped the second.

Charring and volatilization of carbohydrates before they reached the flame was a source of trouble with materials such as raisins. In seeking to obviate this condition, a method of dropping separate pellets of the material to be burned into a combustion dish was devised. It proved to be so successful that the first method was abandoned completely. The improved apparatus is shown in figure 1.

Fruit was divided as necessary, weighed, cut into thin slices, and dried in deep porcelain dishes on a water bath until most of the moisture was gone. The time required varied greatly, but 6 to 12 hours sufficed for most materials. Drying was then completed by placing the sample in a vacuum oven at  $80^{\circ}$ – $90^{\circ}$  C for a few hours. Nonhygroscopic material was then ground through a meat grinder with the fine disk attached and pellets weighing about 1 gram each were wrapped in onionskin paper. Ripe grapes absorb moisture from ordinary air with great rapidity and become sticky to handle. This may be avoided by grinding and wrapping in a coldroom in which the humidity is low.

The process of burning dried material is described best with reference to figure 1, which shows the main parts of the apparatus. A first pellet is placed in the combustion dish *A*, and a tail of the wrapping paper is ignited. Chamber *C*, with more pellets in the side arm, is placed in posi-

tion and the oxygen flow and suction are started. These must be so regulated that combustion is clean, no gas escapes through the water seal *K*, and frothing does not become excessive in the absorbers *L* and *L'*. A flowmeter in the oxygen inlet tube is very useful. Each pellet should be burned to a white ash before the next one is introduced. This is done by

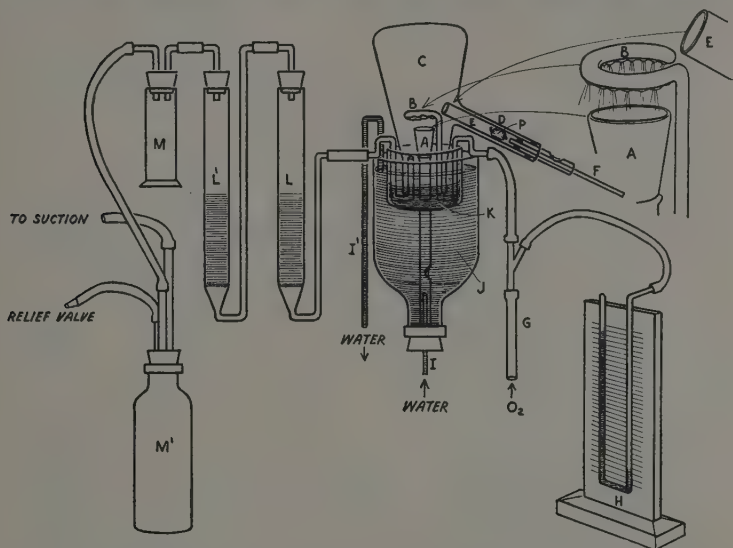


Fig. 1.—Apparatus for combustion of samples: *A*, combustion dish; *B*, ring burner made of Pyrex glass; *C*, combustion chamber; *D*, side arm; *E*, cartridge holder; *F*, rod; *G*, tube for introduction of oxygen; *H*, flowmeter; *I*, *I'*, tubes for circulating water through outer jacket, *J*; *K*, water seal; *L*, *L'*, absorbers; *M*, *M'*, traps; *P*, pellet in holder.

pushing in the rod *F* until the next pellet drops into the dish. If one loading of the side arm is not sufficient, more pellets may be placed in it by withdrawing rod *F*. The temperature of the dish remains high enough for some time to ignite the next pellet. Excessive heating of the water seal and of the inlet and outlet tubes is avoided by maintaining a continuous flow of tap water through the cooling jacket *J*, which is simply the upper part of a large acid bottle.

The success of a combustion is indicated by the color of the interior of the flask and of the water in the absorbers. If these remain relatively colorless, the organic matter is nearly all oxidized. On the other hand, much tarry or resinous matter will prevent the successful isolation of selenium.

When a burning is ended, the combustion chamber is allowed to cool while the contents of the absorbers are washed into a 250-cc distillation flask which connects by means of a ground-glass joint to a condenser. The inside of the combustion chamber *C* is rinsed with a small amount of hot nitric acid, then by distilled water, the combustion dish is dropped into the distillation flask, any ash which adheres to the inlet or outlet tubes is scraped into the water seal, and the tubes are washed with a very small quantity of hot nitric acid to dissolve any selenium on them. Lastly, the

TABLE 3  
RECOVERY OF SELENIUM ADDED TO PLANT MATERIAL

Selenium added		Plant material	Selenium found	Per cent recovery
Amount	Form			
7			7	<i>per cent</i>
25	Selocide	Grapes.....	23	92
25	Selocide	Grapes.....	22	88
32	Selenite	Citrus pulp.....	26	81
55	Selocide	Citrus rind.....	58	105
60	Selenite	Citrus pulp.....	57	95
62	Selenite	Grapes.....	45	73
64	Selocide	Citrus rind.....	57	89
65	Selenite	Grapes.....	52	80
66	Selocide	Citrus pulp.....	57	86
72	Selenite	Citrus rind.....	61	85
76	Selenite	Citrus rind.....	78	103
79	Selenite	Grapes.....	69	87
79	Selenite	Barley straw.....	77	97
82	Selenite	Grapes.....	71	87
82	Selenite	Citrus pulp.....	85	104
158	Selenite	Citrus pulp.....	146	92

water seal with everything which has fallen into it is transferred to the distillation flask. A little hydrobromic acid is added first to destroy the excess nitric acid. Then bromine and hydrobromic acid are added, and the selenium is distilled as in the method of Robinson *et al.* (1934). The distillate is treated with sulfur dioxide and hydroxylamine hydrochloride to precipitate the selenium. The rest of the process is as described for the precipitation, reoxidation, and titration of pure selenite solutions.

The results obtained in sixteen runs with varying amounts of selenium added to different plant materials are shown in table 3. The recovery with grapes (average 84.5 per cent) was distinctly lower than with citrus (average 94.5 per cent). This is due at least in part to the fact that this portion of the work with grapes was done before the improved method of combustion was used, and in several cases combustion was very poor. While, on the basis of these results, correction factors might be applied

to the analyses of material from the field, they would have no effect whatsoever upon the significance of the results and hence are not used. Data in the subsequent tables often is given to two decimal places as calculated from the titration figures. This undoubtedly exceeds the accuracy of the results, but it is felt that they are known to the first decimal.

Through the courtesy of H. G. Byers, a sample of narrow-leaf milk vetch, *Astragalus pectinatus*, from Kansas, was secured. The average selenium content from several closely agreeing analyses was found to be 1,407 p.p.m. Byers (1936) has reported this sample to contain 1,750 p.p.m. Se. The finely ground sample had a very strong repulsive odor, which is characteristic of plant materials that lose a volatile selenium compound when dried (Beath, Eppson, and Gilbert, 1937); and since about a year elapsed between analyses in the two laboratories, a decrease in selenium content would be expected. The good agreement of the repeated analyses in the present work is an indication that the method is applicable to highly seleniferous plant material.

*Analysis for Selenium in Soil.*—For the separation of selenium from soils, the bromine-hydrogen bromide distillation method of Robinson *et al.* (1934) was used. Since this is a recognized method, only a few tests with known amounts of added selenium were made to ensure that the technique was correct. The distillate which contained selenium as selenious bromide was put into a boiling water bath, saturated with sulfur dioxide, and treated with hydroxylamine hydrochloride. The separation of the free selenium and its reoxidation and titration were carried out as described earlier.

## SELENIUM IN AND UPON GRAPES AND CITRUS

*Grapes*.—Numerous sprays containing Selocide were applied to grape vines during the months of May, June, and July. Fruit was harvested in September and analyzed for selenium content. The results indicated that considerable variation occurred in the distribution of selenium. The following data cover the more important spray mixtures used. All results in this section are expressed in terms of fresh weight.

Treatment	Date of spraying	Se, p.p.m. (fresh weight)
Selocide 1:400 + 4 oz. blood-albumin spreader	June 9	0.5
to 100 gals.....	July 7	2.5
Selocide 1:600 + 4 oz. blood-albumin spreader	June 23	0.3
to 100 gals.....	July 7	1.8
Selocide 1:400 + 8 oz. Fluxit to 100 gals.....	July 7	0.7
Selocide 1:600 + 8 oz. Fluxit to 100 gals.....	July 7	0.7
Selocide 1:600 + liquid soap 1:400.....	June 23	0.8
Selocide 1:800 + tank-mix oil 1:400.....	May 22	1.0
Selocide 1:800 + 4 oz. Fluxit, 4 oz. blood-albumin spreader, and 5 lbs. sulfur to 100 gals.....	July 12	0.5
Selocide 1:100 + 4 oz. Fluxit, 4 oz. blood-albumin spreader, and 5 lbs. sulfur to 100 gals.....	July 12	0.4
None.....	.....	0.11
	.....	0.08
	.....	0.20
	.....	0.06
	.....	Av. 0.11

The different wetting powers of the sprays and the varying stability of Selocide in them make impossible any close correlation between concentration of selenium in the sprays and the amounts left upon the grapes. However, it appears that a considerable deposit resulted from the more concentrated sprays and that early applications left but little selenium, probably because both fruit growth and loss by weathering are marked in the early part of the season.

A few preliminary experiments were carried out to test the absorption by grapes of selenium applied to the soil. In each of these tests, on March 15, 2 gallons of solution containing the specified amount of a selenium preparation were poured about a vine over a circle of about 3-foot radius. All fruit was picked for analysis on July 24. The results showed that the grapes from vines treated with 1 pint Selocide (23.6 grams Se) or with 36.7 grams of sodium selenite (17.6 grams Se) contained 0.05 p.p.m. Se, which was the same as that in grapes from an untreated vine. The application of 5 pints of Selocide (118.2 grams Se) resulted in the occurrence



of 0.16 p.p.m. Se in the fruit. Evidently no marked absorption occurred during the interval March 15 to July 24.

*Citrus.*—Selocide sprays have been applied at intervals to certain areas of orange and lemon groves since 1932. During the spring and summer of 1935, samples of fruit from several of these groves were analyzed for selenium content. The results are given in table 4.

In an attempt to determine if selenium is absorbed from the soil after large amounts of Selocide have been applied to the ground about lemon trees, 520 cc of Selocide in 22 gallons of water were applied to a depth of 18 inches in a 12-foot square on September 3, 1933. This amounted to 3 p.p.m. Se in the first 18 inches of soil. Fruit picked on March 10, 1935, contained 0.02 p.p.m. Se in the rind and none in the pulp. A similar treatment, except that 1,732 cc Selocide were used in 34 gallons of water (10 p.p.m. Se in the soil) resulted in the following amounts of selenium in the fruit picked March 10, 1935: rind, 0.31 p.p.m.; pulp, 0.05 p.p.m. Fruit from an untreated tree in the same grove had 0.07 p.p.m. Se in the rind and none in the pulp, and that from another untreated tree: rind, 0.06 p.p.m.; pulp, 0.03 p.p.m. Se.

*Effects of Several Years' Spraying upon Selenium Content of Soil and Fruit.*—The data given in the two preceding sections indicated that but little selenium occurs in citrus fruit or grapes as the result of ordinary spraying with Selocide over short periods. Since the material had been applied to certain groves and vineyards for several years, both soil and fruit from these localities were analyzed to obtain information on the distribution of selenium in the soil and its absorption into fruit.

During the summer of 1937, fruit and soil samples were taken from eight citrus groves which had been sprayed to varying extents with Selocide. Similar samples were taken from other parts of the same or neighboring groves which never had been treated with selenium. In each case, the corresponding samples were from localities of the same soil type and treated by the same irrigation and other cultural practices. The exterior of each fruit used for analysis was washed in nitric acid to remove any adhering selenium. A preliminary account of the analytical results has already been published (Hoskins, 1938). The complete data are given in table 5.

The soils were classified by M. R. Huberty, Associate Irrigation Engineer in the Experiment Station. Additional information concerning their origin has been furnished by C. F. Shaw, Soil Technologist in the Experiment Station, Berkeley, as follows:

The Altamont soils were formed by the weathering in place of marine sandstones

TABLE 4  
SELENIUM OCCURRING IN AND UPON CITRUS AS A RESULT OF SPRAYING WITH SELOCIDE

Lot No.	Treatment		Remarks: Maturity and variety of fruit and time from last spraying to analysis	Selenium (on basis of fresh weight)		
	Material	Date		Rind	Pulp	Whole fruit
Oranges						
1	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	Summer 1933	Mature Valencias; 9 months.....	<i>p. p. m.</i> 0.27	<i>p. p. m.</i> 0.06	<i>p. p. m.</i> 0.11
3a	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	Summer 1934	Mature Valencias; 9 months.....	0.06	0.03	0.04
3b	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	June 18, 1934	Half-grown Valencias*.....	0.00	0.00	0.00
3c	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	June 18, 1934	Mature Washington Navels; 9 months.....	0.05	0.03	0.04
5a	Selocide 1:800+ $\frac{1}{2}$ per cent oil and 3 lbs. cryolite.....	July 4, 1934	Mature Valencias; 9 months.....	0.32	0.07	0.12
5b	Selocide 1:800+ $\frac{1}{2}$ per cent oil and 3 lbs. cryolite.....	July 4, 1934	Green Valencias*.....	0.00	0.02	0.01
6a	{ Selocide 1:800+ $\frac{1}{2}$ per cent lime-sulfur.....	Aug. 22, 1934	Mature Valencias; 9 months.....	0.18	0.00	0.05
	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil and 3 lbs. cryolite.....	June 1, 1934				
6b	{ Selocide 1:800+ $\frac{1}{2}$ per cent lime-sulfur.....	Aug. 22, 1933	Green Valencias*.....	0.01	0.05	0.04
	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil and 3 lbs. cryolite.....	June 1, 1934				
10	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	Dec. 9, 1933	Mature Valencias*.....	0.00	0.02	0.02
	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	July 7, 1934				
13c	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil and $\frac{1}{2}$ per cent lime-sulfur.....	Mar. 30, 1935	Mature Valencias; 6 weeks.....	0.8	0.03	0.25
	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	July 7, 1934	Put through regular packing-house treatment (no waxing); 38 per cent of Se removed from rind.....	0.5	....	....
13d	{ Selocide 1:800+ $\frac{1}{2}$ per cent oil and $\frac{1}{2}$ per cent lime-sulfur.....	March 30, 1935	Mature Valencias; 1 week.....	0.41	0.01	0.11
14a	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	May 7, 1935	Put through regular packing-house treatment (no waxing); 68 per cent of Se removed from rind.....	0.13	....	....
14b	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	May 7, 1935	Mature Navels; 10 months.....	0.05	0.03	0.03
			Put through regular packing-house treatment (no waxing); 20 per cent of Se removed from rind.....	0.04	....	....
15a	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	July, 1934				
15b	Selocide 1:800+ $\frac{1}{2}$ per cent oil.....	July, 1934				

Grapefruit						
		Dec. 9, 1934	4 months; red Se visible on lower surface.....	p.p.m. 0.83	p.p.m. 0.02	p.p.m. 0.46
5	Selocide 1:600+ $\frac{1}{4}$ per cent oil.....					
Lemons						
2	Selocide 1:800+ $\frac{1}{4}$ per cent lime-sulfur.....	Nov. 7, 1934	Mature; 4 months.....	p.p.m. 0.72	p.p.m. 0.02	p.p.m. 0.26
2a	Selocide 1:800+ $\frac{1}{4}$ per cent lime-sulfur.....	Nov. 7, 1934	Mature; 4 months.....	0.80	0.01	0.27
5	None.....	Oct. 31, 1932	Half-grown.....	0.06	0.03	0.04
	Selocide 1:600.....	Mar. 21, 1933				
9	Selocide 1:600.....	July 8, 1934	Mature; 3 months.....	1.95	0.21	0.63
	Selocide 1:800.....	Mar. 30, 1935				
	Selocide 1:800.....	Oct. 10, 1935				
2	Selocide 1:800+ $\frac{1}{4}$ per cent oil.....	July 7, 1934	Mature; 8 months.....	0.16	0.02	0.06
7	Selocide 1:800+ $\frac{1}{4}$ per cent oil.....	April 11, 1934	Mature; 11 months.....	0.06	0.01	0.03
8	Selocide 1:600+ $\frac{1}{4}$ per cent oil.....	Dec. 9, 1933	Mature*.....	0.00	0.06	0.03
	Selocide 1:800+ $\frac{1}{4}$ per cent oil.....	July 7, 1934	Mature; 2 months.....	1.30	0.10	0.52
13a	Selocide 1:800+ $\frac{1}{4}$ per cent oil and $\frac{1}{4}$ per cent lime-sulfur.....	Mar. 30, 1935				
	Selocide 1:800+ $\frac{1}{4}$ per cent oil.....	July 7, 1934	Put through regular packing-house process; 29 per cent of Se removed from rind.....	0.90	....	....
13b	Selocide 1:800+ $\frac{1}{4}$ per cent oil and $\frac{1}{4}$ per cent lime-sulfur.....	Mar. 30, 1935				

\* Fruit not on tree when spray was applied to tree.

TABLE 5  
RESULTS OF ANALYSIS OF FRUIT AND SOIL FROM CITRUS GROVES, 1937

Plot	Fruit	Soil type	Applications of Selsolcide	Se in soil				Se in fruit		
				0-6 in.	6-12 in.	12-24 in.	24-36 in.	Skin*	Pulp*	Whole fruit (av.)
				p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
A	Lemons	Yolo clay loam.....	{ October, 1932, 1:600..... March, 1933, 1:600..... July, 1934, 1:600..... March, 1935, 1:800..... October, 1935, 1:800..... January, 1936, 1:800..... }	2.08	1.29	0.76	0.29	{0.09 0.08	{0.07 0.08	0.08
AA	Valencia oranges	Yolo clay loam.....	None.....	0.43	0.22	0.23	0.23	{0.17 0.09 0.17 0.10	{0.09 0.17 0.10	0.12
B	Valencia oranges	Hanford fine sandy loam.....	Five during 1933 to 1935 at 1:600 and 1:800, last in Apr. or May, 1936.....	1.28	0.42	0.26	0.27	{0.53 0.42	{0.11 0.03	0.17
BB	Valencia oranges	Hanford fine sandy loam.....	None.....	0.51	0.24	0.27	0.22	{0.04 0.09	{0.04 0.03	0.04
C	Lemons	Hanford stony sandy loam.....	{ October, 1934, 1:800..... March, 1935, 1:800..... January, 1936, 1:800..... February, 1936, 1:800..... November, 1936, 1:800..... }	2.16	0.67	0.60	0.39	{0.31 ....	{0.07 0.05	0.12
CC	Lemons	Hanford stony sandy loam.....	None.....	0.39	0.23	0.25	0.25	{0.14 0.11	{0.04 0.14	0.10
D	Valencia oranges	Hanford gravelly sandy loam.....	{ January, 1935, 1:800..... January, 1936, 1:800..... May, 1936, 1:800..... }	1.50	0.68	0.41	0.34	{0.26 0.18	{0.12 0.12	0.16

DD	Valencia oranges	Hanford gravelly sandy loam.....	None.....	0.41	0.25	0.23	0.29	{ 0.09 0.12 }	{ 0.03 0.02 }	0.05
E	Valencia oranges	Altamont clay.....	{ July, 1933, 1:600..... June, 1934, 1:800..... April, 1936, 1:600..... }	0.48	0.26	0.24	0.20	{ 0.37 0.08 0.08 }	{ 0.05 0.10 }	0.10
EE	Valencia oranges	Altamont clay.....	None.....	Composite, 0.35				{ 0.00 0.18 }	{ 0.01 0.07 }	0.05
F	Lemons	Yolo silt loam.....	{ September, 1933, 1:800..... August, 1934, 1:800..... March, 1935, 1:800..... October, 1935, 1:800..... November, 1935, 1:800..... }	1.07	1.10	0.86	0.86	{ 0.12 0.25 }	{ 0.02 0.03 }	0.07
FF	Lemons	Yolo silt loam.....	None.....	Composite, 0.60				{ 0.13 0.03 0.15 }	{ 0.01 0.02 }	0.05
G	Lemons	Yolo fine sandy loam.....	Spring of 1936, 1:800.....	0.37	0.15	0.18	0.21	{ 0.34 0.12 0.15 0.29 }	{ 0.03 0.04 }	0.10
GG	Lemons	Yolo fine sandy loam.....	None.....	Composite, 0.12				{ 0.04 0.12 0.01 }	{ 0.01 0.01 }	0.04
H	Valencia oranges	Yolo loam.....	{ June, 1934, 1:1,000..... August, 1934, 1:800..... }	0.85	0.72	0.54	0.31	{ 0.08 0.10 0.04 }	{ 0.01 0.04 }	0.05
HH	Valencia oranges	Yolo loam.....	None.....	Composite, 0.31				{ 0.03 0.09 }	{ 0.02 0.02 }	0.04

\* Data are for duplicate or replicate tests on fruit from the various plots.



and shale rocks, in this case of marine origin, which have not been leached to any great extent.

The Hanford soils were derived from stream deposits on alluvial fans and have their origin from granitic rocks.

The Yolo soils were formed from stream deposits on alluvial fans and flood plains and are derived from a variety of sandstones and shales which are mainly of marine origin.

The results with Thompson Seedless grapes are given in table 6.

TABLE 6  
RESULTS OF ANALYSIS OF FRUIT AND SOIL\* FROM VINEYARDS, 1937

Plot	Application of Selocide	Se in soil				Se in grapes (ripe)	
		0-6 inches	6-12 inches	12-24 inches	24-36 inches	Unwashed	Washed
		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
1	None.....	0.32	0.25	0.24	0.23	$\begin{cases} 0.11 \\ 0.06 \end{cases}$	$\begin{cases} \dots \\ \dots \end{cases}$
2	$\begin{cases} (1933, 1:400) \\ (1934, 1:400) \\ (1935, 1:600) \\ (1936, 1:600) \\ (1937, 1:600) \end{cases}$ .....	1.00	0.49	0.41	0.33	$\begin{cases} 1.80\dagger \\ 0.80 \end{cases}$	0.67
3	$\begin{cases} (1933, 1:400) \\ (1934, 1:400) \\ (1935, 1:600) \\ (1936, 1:600) \\ (1937, 1:600) \end{cases}$ .....	1.51	0.63	0.61	0.30	$\begin{cases} 0.61 \\ 0.65 \end{cases}$	0.26
4	$\begin{cases} (1933, 1:400) \\ (1934, 1:400) \end{cases}$ .....	....	....	....	....	0.14	....
5	$\begin{cases} (1935, 1:600) \\ (1936, 1:400) \end{cases}$ .....	....	....	....	....	0.23	....

\* These soils were all clay loams, but the particular types have not been determined as yet.

† The wide variation between these two figures is an indication of unevenness in application of the spray. Free selenium could be seen upon the grapes which had the larger residue.

## THE NATURAL OCCURRENCE OF SELENIUM

The results reported for citrus and grapes in the foregoing section indicate that the amounts occurring in or upon these products as the result of the proper use of Selocide are a few hundredths of a part per million in most cases. While this may be taken as an indication that a hazard either to the plants or to animals or humans is not likely to result from the use of this spray material, an examination of the available information on the natural exposure of plant and animal life to selenium and

its effects thereon is advisable before conclusions are drawn. In the following sections, published data are collected and certain experimental results of the present investigation are mentioned.

*Abundance of Selenium.*—Although selenium is ordinarily considered to be a comparatively rare element, it ranks about fiftieth in abundance among the elements making up the crust of the earth (Noddack and Noddack, 1930, 1934) and is more abundant than iodine and nearly as common as arsenic. Its close association with sulfur is indicated by the fact that it was first found in the flue dust of a sulfuric acid plant. Chemically, selenium and sulfur are much alike, and they form analogous series of compounds. According to Goldschmidt and Hefter (1933), sulfur is oxidized somewhat more readily than selenium, so that in the course of geologic time, a larger fraction of the selenium tends to remain in place as the free element, whereas sulfur is oxidized to soluble form and removed. This condition, together with the strong adsorption of selenium by hydrated iron oxide (Goldschmidt and Strock, 1935), largely explains the changes from the original selenium: sulfur ratio which have come about in various parts of the earth's crust.

*Selenium in Soils.*—Selenium has been found to occur in practically all soils which have been examined for its presence. The concentration varies widely but may be correlated in a general way with the geological origin of the various soils (Byers, 1935; Strock, 1935). Thus, that from sedimentary deposits contains most of the selenium which has been carried into the sea by rivers. Ocean water is low in selenium content—for example, 0.004 p.p.m. near Helgoland—and the Se:S ratio in salt deposits from ancient seas is low (Goldschmidt and Hefter, 1933).

An examination of Hawaiian soils led Byers, Williams, and Lakin (1936) to the conclusion that the selenium therein was of volcanic origin. They suggested that the original source of selenium now occurring in many sedimentary formations in the central United States was volcanic gases and dust from the numerous volcanoes of the Upper Cretaceous period. After entering the oceans, which at that time covered most of the present area of this country, the selenium was soon combined with iron compounds and carried to the bottom. The various sedimentary layers differ in their content of selenium according to the volcanic activity at the time of their deposition. That known as the Pierre shale, which extends over parts of nearly a dozen states in the Midwest and Rocky Mountain region, is exceptionally rich in this element.

In soils from magmatic rocks, the selenium is due mainly to that which was originally associated with the metallic sulfides. Beath, Gilbert, and Eppson (1937) have suggested that intrusions of such materials may

account for the selenium now present in the sedimentary rocks and their derived soils in Wyoming.

A further factor influencing the distribution of selenium is the dissolving of its compounds in underground waters and passage as solutes to locations favorable for reprecipitation.

The net result of all these processes is that the distribution of selenium is world-wide but very far from uniform. The free element is very insoluble, but at least the red allotropic form is converted in the presence of water and mild oxidizing agents into soluble combined states (Montignie, 1934; Calcagni, 1935); and also, certain bacteria are able to effect the oxidation of the free element (Lipman and Waksman, 1923).

The compounds of selenium are, on the whole, at least as soluble as those of sulfur, and leaching by either naturally or artificially applied water may be expected to reduce the selenium content of a soil provided the drainage is such that percolation of water through the soil occurs readily. This effect will not necessarily be shown immediately but regions of habitually plentiful rainfall or long-continued heavy irrigation must eventually come to the condition of minimum soluble selenium and usually low total selenium in the soil. This is particularly true of sandy soils, less so with heavy clays through which water moves with difficulty. The Belle Fourche Irrigation Project in South Dakota has been cited by Byers (1935) as an example of the effect of irrigation for twenty-five years. In this area, the selenium content of the soils is only a little less than that in neighboring unirrigated districts, but that of the vegetation was uniformly low.

The forms in which selenium occurs in the soil have not been investigated with thoroughness, but they may be divided in general into organic and inorganic. On account of the completeness with which hydrated iron oxide removes selenium from solution, one may reasonably suppose that some of it is present as a very insoluble iron selenide or basic selenite (Williams and Byers, 1936). The more soluble inorganic portion may be present as calcium selenite or selenate. Electrodialysis experiments (Franke and Painter, 1937) have shown that selenium added to soil as sodium selenite in large part became nondialyzable. Previous destruction of the organic matter in the soil by heating to 500° C did not prevent binding of added selenium. Selenate is much less easily fixed by the soil. The probable nature of the organic compounds of selenium and their special significance with regard to plant absorption will be considered later.

Only very fragmentary data are available regarding the selenium content of soils in various parts of the world, and, in fact, only in the United

States has a start been made toward a survey of the situation. The results summarized by Byers (1935, 1936) show that selenium occurs in South Dakota, Nebraska, Kansas, Oklahoma, Montana, Wyoming, Colorado, Utah, New Mexico, Arizona, and the Hawaiian Islands. In South Dakota every sample taken in an area of about 500,000 acres contained selenium in amounts varying from a trace to 40 p.p.m. The highest selenium content so far reported for a soil in the United States (Logan County, Kansas) is 82 p.p.m., but only 10 feet away it was but 16 p.p.m. Such varia-

TABLE 7  
SELENIUM CONTENT OF SOILS AT VARIOUS DEPTHS

Depth	Niobrara clay lime*		Selenium in "poison area" in Colorado†
	Selenium in Horizon A	Selenium in Horizon B	
<i>inches</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
0-12.....	6.0	1.0	1.4
12-24.....	1.0	0.7	1.1
24-36.....	0.8	0.5	2.6
36-48.....	1.0	0.5	6.4
48-60.....	0.8	0.5	17.8
60-72.....	0.8	0.7	4.7

\* From Byers (1936), p. 39.

† From Beath, Eppson, and Gilbert (1937).

tions occur not only from point to point but in profiles. Thus Niobrara clay lime in Kansas and a known "poison area" in Colorado showed considerable variation of selenium with depth (table 7).

A few data for Hawaiian soils are particularly interesting because they range in selenium content from 0.4 p.p.m. to 26.0 p.p.m. with no decrease in the regions in which there are as much as 120 inches of rain yearly (Byers, Williams, and Lakin, 1936). The explanation offered for this absence of leaching is that the soils contain much iron, and hence the selenium probably is held in an insoluble complex combination. The idea that selenium will be low in regions having 20 inches or more of rain (Wilcox, 1935) is thus shown not to be applicable everywhere, but it is true that abundant rainfall leads to low amounts of available selenium.

*Selenium in Water.*—An indication of the effect of increased drainage in removing soluble selenium from the soil is given by the analysis of certain drainage waters of the Belle Fourche Irrigation Project (Byers, 1935). The water from a tile drain installed in 1916 and in use ever since contained 0.08 p.p.m. Se, whereas that in a drain installed in 1933 for the purpose of improving the movement of water from a certain area contained 1.2 p.p.m. Further illustration of the effect of irrigation is af-

forded by analyses of water in drainage ditches which empty into the Colorado River in Colorado. The selenium content varied from 0.32 to 2.68 p.p.m. (Williams and Byers, 1935). Above the discharge point of these ditches, the water of the Colorado River was free of selenium. Several tributaries were contaminated in a similar way. The result was the presence of 0.03 p.p.m. Se in the Colorado River near Grand Junction. Since irrigation has been practiced on a large scale in these districts for a comparatively short time, it is to be expected that large amounts of soluble selenium will be removed until that remaining is greatly reduced.

The water of shallow wells in the seleniferous areas of Nebraska and South Dakota has been found to contain as much as 0.2 p.p.m. Se, but deep well water is practically free of it. Similarly 0.56 p.p.m. Se was found in the water of a shallow well near Fallon, Nevada, but a deep artesian well nearby contained none (Byers, 1936). Of 44 samples of drinking water from wells in South Dakota and Nebraska examined by Smith and Westfall (1937) 23 per cent contained selenium in amounts from 0.05 to 0.33 p.p.m. No definite relation between depth and selenium content could be found. A so-called "poison spring" in South Dakota was found to have 0.4 p.p.m. Se in its water (Miller and Byers, 1935). It is interesting to note that selenium at a concentration of 0.2 p.p.m. was found in the medicinal waters of La Roche-Posay in the department of Vienne, France (Taboury, 1909).

*Selenium in Plants.*—Probably so reactive an element as selenium should be expected to be absorbed to varying extents by different kinds of plants. The facts so far determined reveal a truly amazing selectivity. On the basis of their tendency to accumulate selenium, plants may be classified into three groups, corresponding to (a) high, (b) moderate, and (c) slight absorption (Beath, Eppson, and Gilbert, 1935; Miller and Byers, 1937).

In group *a* are included certain species of *Astragalus* (family Leguminosae), especially *A. racemosus* and *A. bisulcatus* (poison vetch); *Stanleya pinnata* and *S. bipinnata* (family Cruciferae), and *Xylorhiza Parryi* (family Compositae). These plants grow more vigorously on seleniferous soil and may accumulate the element to the extent of a 1,000 p.p.m. or more: for example, Byers (1935, 1936) gives examples of *Astragalus bisulcatus* containing 2,000 to 4,000 p.p.m. Se growing on soils which contained from 0.8 to 3.0 p.p.m. Se. The highest selenium content so far reported (14,920 p.p.m.) is for a sample of *A. racemosus* growing on a Pierre shale which had 22.7 p.p.m. Se. No sign of injury was evident. Possibly the element plays a rôle in the physiology of these plants (Trelease and Trelease, 1938).



An extremely important result of the growth of such plants in a seleniferous region is the conversion of selenium from a relatively insoluble condition to one in which it is readily absorbed by many plants other than those of group *a*. Hence the latter have been called "selenium converters," or "indicators."

Not all species in a genus behave alike in selenium absorption. This contrast in the behavior of closely related plants is well illustrated by *Astragalus*. Thus, in five plots having a mean soil content of 2.1 p.p.m. Se, *A. bisulcatus* averaged 1,250 p.p.m. Se and *A. missouriensis* 3.1 p.p.m. Se. Further data are given by Byers and Knight (1935). Similar differences have been found in other families and genera.

In group *b* are included *Aster Fendleri* and *A. multiflorus* (family Compositae), several species of *Atriplex* (family Chenopodiaceae), and the ordinary cereals (family Gramineae). These plants are not able to concentrate large amounts of selenium from ordinary seleniferous soil, but if they grow near the spot at which a selenium-converting plant has grown, they may accumulate the element very markedly.

In an experiment reported by Beath, Eppson, and Gilbert (1937), wheat was grown on Steele shale soil through which green *Astragalus bisulcatus* was dispersed in the upper foot in such quantity that the average concentration of selenium was 10 p.p.m. At the conclusion of the growing season, the wheat contained selenium as follows: heads, 95 p.p.m.; stem and leaves, 123 p.p.m.; roots, 107 p.p.m. Another sample of the same wheat grown upon the same shale without the addition of the *Astragalus bisulcatus* contained only a trace of selenium. In the same experiment, wheat was raised on the same soil to which potassium selenite had been added at the rate of 10 p.p.m. selenium. In the matured wheat, selenium was present as follows: heads, 19 p.p.m.; stem and leaves, 17 p.p.m.; roots, 36 p.p.m. Thus it was shown that selenium from decomposed "converter plants" is much more readily taken up by other plants than is selenium in inorganic combination, although the difference may be due to failure of the selenium from plants to be rendered insoluble by reaction with metallic salts of the soil, for example, those of iron. Further consideration of the availability of selenium will be given in a later section.

Plants of group *b* are usually affected adversely by large amounts of selenium in their tissues. Thus Hurd-Karrer's (1935) data show that wheat grown on soil treated with sodium selenite showed injury when its content of selenium was from 300 to something over 600 p.p.m., calculated on an air-dry basis. Analysis of wheat from various parts of the world, including Argentina, Australia, Canada, Hungary, Mexico, New

Zealand, South Africa, and Spain (Robinson, 1936) showed selenium to be present in every sample in amounts from 0.1 to 1.9 p.p.m. The samples were obtained from the respective marketing centers and hence represent average conditions for large areas. Undoubtedly, wheat from restricted localities in other countries contains high amounts of selenium just as in the case of certain areas in the United States. Unfortunately, in the manufacture of flour, the toxic portion of grain is not lost: Horn, Nelson, and Jones (1936) found the toxic principle to be uniformly distributed in the milled fractions. Smith and Westfall (1937) have reported analyses of cereal grains from Nebraska and South Dakota which may be summarized as follows:

Grain	Selenium, p.p.m.	
	Minimum	Maximum
Wheat .....	1.15	18.8
Corn .....	1.00	14.9
Barley.....	1.65	5.75
Oats .....	2.00	10.00
Rye .....	0.87	3.80

Hurd-Karrer (1935) has reported that mustard absorbs large quantities of selenium from soil containing 5 p.p.m. Se as sodium selenate. Accordingly, it was of great interest to determine if the common mustard, *Brassica campestris*, which comprises a large portion of the covercrop in the citrus district of California, absorbs much selenium from the soil of treated groves. Samples were selected from beneath and around the trees and found to contain from 1 to 4.7 p.p.m. Se, with no consistent difference between treated and untreated plots. This is a further indication that the residue of selenium from Selocide is not readily absorbed by plants. Of course, the possibility remains that some other plant species grown now or in the future in the citrus or vineyard districts of California, will prove to be a "selenium converter." A plant survey should be made for these districts similar to those already made in South Dakota, Nebraska, Wyoming, etc.

The plants of group *c*, which have a limited tolerance for selenium and are able to absorb but little of it even from highly seleniferous soils, include the gamma grasses, *Bouteloua gracilis* and *B. curtipendula*, buffalo grass, *Buchloe dactyloides*, and numerous other native range plants (Beath, Eppson, and Gilbert, 1935). Unfortunately, the data available as yet are largely limited to plants of the Rocky Mountain and Great Plains regions.

The question of absorption of selenium by alfalfa has received some attention. Since alfalfa is a member of the same family as *Astragalus*, it might conceivably be of the "selenium converter" type. From a clay loam

soil to which 4 p.p.m. Se had been added as sodium selenate, Hurd-Karrer (1937) found alfalfa to collect 220 p.p.m. Se (air-dry basis). Byers (1936) found 200 p.p.m. Se in a sample from an irrigated field whose soil contained but 0.7 p.p.m. Other samples on various other soils had up to 40 p.p.m. Several samples of alfalfa from soils of 0.5 p.p.m. Se contained none. Beath, Eppson, and Gilbert (1935) found only a few parts per million of selenium in alfalfa growing on seleniferous shales but were able to give more to samples of the plant by irrigating them with aqueous extracts of *Astragalus bisulcatus*. No sample of alfalfa from South Dakota analyzed by Moxon (1937) contained more than 10 p.p.m. Se. In the present investigation, dried alfalfa from the San Joaquin Valley was found to contain only a trace of selenium, whereas in the soil on which it was grown there were 0.4 p.p.m. The evidence indicates that alfalfa does not absorb really large amounts of selenium from soils containing it to the extent of several parts per million. Probably it belongs to group *b*, whose members take up large amounts of selenium only when the element has been rendered available by previous incorporation in a "selenium-converter plant." In the present investigation, melilotus clover was grown by D. R. Hoagland of the Plant Nutrition Division on soil to which Selocide had been added at the rate of 60 kg Se per acre. The dry plants were analyzed by P. L. Kirk of the Biochemistry Division and found to contain 0.3 p.p.m. Se. Apparently this clover does not absorb selenium readily.

Comparatively little data are at hand regarding absorption of selenium from the soil by truck crops. Plants grown on Keyport clay loam to which sodium selenate had been added to give 5 p.p.m. Se contained the following amounts of selenium in their leaves (Hurd-Karrer, 1937) :

Plant	Selenium, p.p.m.
Cabbage, young.....	344
Cabbage, old .....	180
Broccoli .....	220
Turnip .....	240
Pea .....	60
Corn .....	60
Spinach .....	50
Lettuce .....	50

Byers (1935) found the following amounts :

Plant	Selenium, p.p.m.
Turnips .....	25
String Beans .....	2
Lettuce.....	7
Cabbage .....	100

Unfortunately, no information concerning the soil was given. Several kinds of vegetables, grasses, and cereals were grown for four seasons on soils from Steele and Niobrara formations containing 2-4 p.p.m. Se (Knight and Beath, 1937). The highest selenium content of the plants during the fourth season was 3.5 p.p.m. Apparently none of them are "selenium converters." The selenium contents of the edible portions of various vegetables, presumably grown in South Dakota or Nebraska, were reported by Smith and Westfall (1937):

Material	Selenium, p.p.m.	
	Minimum	Maximum
Cucumber .....	0.12	0.55
Potato .....	0.24	0.94
Beet .....	0.32	1.18
Tomato .....	0.18	1.22
Carrot .....	0.43	1.30
Pea, bean.....	0.38	2.04
Cabbage .....	0.23	4.52
Rutabaga .....	1.72	6.00
Onion .....	0.36	17.80

Since these data apparently refer to fresh weight, obviously some of the ordinary vegetables from seleniferous regions may contain as much selenium as the plants which have been classified into group *b*.

Additional data concerning the absorption of selenium by various vegetables was afforded by certain experiments conducted by Hoagland. The results of the analyses, made by Kirk, are summarized in table 8.

Extremely little published information is at hand regarding selenium in the fruit of trees. Byers (1936) found less than 0.1 p.p.m. Se in the pulp and skin of each of eighteen samples of apples from Colorado. The maximum selenium content of the apple seeds was 0.5 p.p.m., and many had only a trace.

The stage of growth may have an important effect upon the content of selenium in various plants. This matter has been investigated by Beath, Eppson, and Gilbert (1937) for several highly seleniferous plants. The following results are typical for those obtained with *Aster commutatis*:

Stage of growth	Month	Selenium, p.p.m.
Initial growth.....	May	590
Prebloom.....	June	273
Full bloom.....	August	233
Seeding.....	November	15

A decrease in selenium content with age cannot be taken as characteristic of all such plants, however, for *Oenopsis condensata* contained the maximum amount at the period of full bloom. Very little information on this

point is available for plants which are important foods for man or animals. Hurd-Karrer (1936) found that wheat plants grown on soil to which sodium selenate had been added at the rate of 30 p.p.m. Se contained 1,120 p.p.m. Se (air-dry basis) when young and but 220 p.p.m. at maturity. Similarly, from nutrient solutions containing 1 p.p.m. Se

TABLE 8  
ABSORPTION OF SELENIUM BY VEGETABLES

Plant	Selenium added to soil		Selenium in resulting plant	
	Form	Amount	Part	Amount
		<i>p.p.m.</i>		<i>p.p.m.</i>
Carrots.....	{ Selocide.....	2	{ Tops	19
			{ Roots	7
	{ Selocide.....	20	{ Tops	25-43
			{ Roots	12
Lettuce.....	{ Selenite.....	1.1	{ Tops	11-18
			{ Roots	2-4
	Selocide.....	20	Tops	26
Peas.....	{ Selocide.....	20	{ Seeds	5-10
	{ Selenite.....	1.1	{ Seeds	10
Tomatoes.....	{ Selenite.....	{ 1	Fruit	10
		{ 10	Vines all died	..
	{ Selenate.....	{ 1	Fruit	28
		{ 10	Vines all died	..
	{ Selocide.....	{ 1	Fruit	6
		{ 10	Fruit	13
		{ 100	Vines all died	..

as sodium selenate, one-month-old wheat plants collected 330 p.p.m. Se, but at maturity the leaves contained 40 p.p.m., the stems 12 p.p.m., and the grain 8 p.p.m.

The various parts of a given species of plant may contain decidedly different concentrations of selenium. The data on wheat (preceding paragraph and p. 147) exemplify this condition. Analysis of a sample of *Astragalus pectinatus* when the seeds were beginning to disperse gave the following results (Beath, Eppson, and Gilbert, 1937):

Material	Selenium, p.p.m.
Pods .....	124
Foliage .....	260
Seeds .....	3,250



*Combination in Which Selenium Occurs in Plants.*—Several workers have attempted to obtain information regarding the manner in which selenium is held in plants by determining its solubility in water or other solvents. Unfortunately, there is poor agreement among the experimenters. Beath, Draize, *et al.* (1934) and Beath, Gilbert, and Eppson (1937) found the selenium in range plants of Wyoming to be freely water-soluble and about 50 per cent of that in wheat to be water-soluble. On the contrary, Horn, Nelson, and Jones (1936) by biological testing found that neither water, alcohol, nor ether removed selenium from wheat. Incidentally, this proves that it cannot be associated with the lipoids. Moxon (1937) also, by a biological method, obtained no evidence that selenium was removed from corn by distilled water or by 5 per cent potassium sulfate solution.

The first suggestion that selenium may be present in plants as a result of replacing sulfur in some organic compound probably was made by Cameron (1880). Among more modern workers, Franke (1934) showed that the toxic material in Dakota wheat and corn followed the protein fraction. Pursuing this work further, Franke and Painter (1936) found little or none of the selenium to occur in the free state or in an inorganic compound. Even after hydrolysis of the proteins to amino acids, the selenium remained in organic combination. Upon extraction of the hydrolysate with chloroform, petroleum ether, alcohol, or benzene, none of the selenium was removed; but it all passed into butyl alcohol within 72 hours. This behavior is like that of the sulfur-containing amino acids cysteine, cystine, and methionine (Painter and Franke, 1935). All cystine precipitants brought down more or less of the selenium present. At least part of the total selenium is in an organic compound whose properties closely resemble those of cystine. The selenium:sulfur ratio in the protein from a sample of toxic wheat was found to be 1:148. In general, the ideas of Franke and his co-workers were confirmed by Horn, Nelson, and Jones (1936). The selenium analog of cystine has been synthesized (Gordon, 1935), but apparently its biological effects have not been reported.

Probably selenium occurs in somewhat different forms in various plants; for Beath, Eppson, and Gilbert (1937) found that it is not volatile from cereals, grasses, or vegetables, but when the "selenium converters" are dried, up to two-thirds of their contained selenium may be lost. In fact, the living plants have an odor which is said to repel foraging animals. Byers and Knight (1935) reported that *Astragalus bisulcatus* can be freed almost completely from selenium by extraction with hot water, and that part of the selenium is volatile with steam. Franke and Painter (1938) showed that even "lethal wheat" becomes less toxic

as it is stored. Such behavior is probably due to loss of a volatile selenium compound.

*Availability of Selenium to Plants.*—Examination of the data of Byers (1935, 1936) indicates that there is no correlation between the concentration of selenium in various soils of the Middle West and Rocky Mountain regions and its concentration in any given species of plant grown on the soils. Since selenium occurs in several forms whose relative amounts doubtless differ in various soils, such a result is to be expected. Lakin, Williams, and Byers (1938) have reported that none of the naturally occurring vegetation from a certain district in Puerto Rico contains as much as 1.0 p.p.m. Se, although the soil content is from 2.5 to 12.0 p.p.m. Se. This situation has forced the above writers to the conclusion that, "Selenium content in soils does not necessarily indicate a dangerous or even slightly harmful situation." They also express the opinion that the selenium left in the soil as a result of the use of Selocide will be available to plants only as the result of slow hydrolytic processes which change it to available forms. In the absence of information concerning the fate of selenium in different combinations added to the soil, conclusions cannot be drawn regarding the true availability of selenium in its various states. The free element has but slight effect, if any, upon wheat (Hurd-Karrer, 1935) but upon a nonseleniferous soil to which free selenium was added to give 25 p.p.m., 3-months-old seedlings of certain *Astragalus* species contained 1,150 p.p.m. Se (Beath, Eppson, and Gilbert, 1937).

Wheat is able to take in much more selenium from added soluble selenate than from selenite (Hurd-Karrer, 1935). This may be the result of a true difference between the hexavalent and tetravalent forms of the element, but more likely it is a consequence of the greater reactivity of the selenite, which may be reduced to the free state or form very slightly soluble compounds—for example, with iron—and hence be less available to plants.

No systematic study of the effect of acid or basic conditions upon the absorption of selenium seems to have been made. Hurd-Karrer (1934, 1935) found no effect upon the toxicity of sodium selenate to wheat seedlings when the pH of soils varied from about 5 to 8. It does not follow that other forms of selenium would be similarly indifferent to pH.

The importance of the nature of the soil in absorption of added selenium by plants has been emphasized by Hurd-Karrer (1935). The physiological effects of sodium selenate upon wheat plants were much more decided in the case of Pierre clay than in that of Keyport clay loam. The latter soil has a relatively higher content of aluminum and iron and hence may be expected to react with or tightly adsorb more of the added

selenium compound. There is little possibility, however, that a soil containing dangerous amounts of selenium may be rendered suitable for use by adding iron, because the cheap iron compounds, such as commercial iron oxide, do not react to any appreciable degree with soluble selenium. The iron compounds which will form insoluble compounds with selenium—for example, iron hydroxide or soluble salts which hydrolyze to form it—are too expensive for agricultural use in the amounts needed. Addition of quartz sand to Keyport clay loam (Hurd-Karrer, 1935) increased the toxic effects upon wheat plants of a given concentration of sodium selenate in the mixture. Knight and Beath (1937) found that Thermopolis shales not only permit but little added selenite to be absorbed by wheat, but even the incorporation of highly seleniferous green *Astragalus bisulcatus* in the soil resulted in little selenium in the wheat grown thereon.

These same authors have reported an interesting difference between the distributions of added inorganic and organic selenium in several Wyoming shale soils. Sodium selenite tends to remain concentrated in the top layers, whereas that from seleniferous plants readily penetrates at least to the 2½-foot depth. That the growth of "selenium-converter plants" over long periods does not result in an accumulation of selenium near the top of the soil is shown by the tables of Byers (1935, 1936) and by the data of Beath, Eppson, and Gilbert (1937).

*Effect of Adding Sulfur.*—The close association of amino acids containing selenium with those containing sulfur has led to the suggestion that plants distinguish between them with difficulty. Hurd-Karrer (1936) has stated:

The only way I have found so far to explain the relationship of sulfur and selenium is to assume that the root cannot tell the difference between them because of their chemical similarity. Assuming that this is true, then the amount of selenium taken in with a given amount of sulfur would depend on the proportionate amounts of the two which are available, the total absorbed being limited. Thus if there is a large excess of sulfur, the root will get relatively little selenium. After the selenium gets in, it can be assumed that the plant proceeds to use it as if it were sulfur, but with serious results. Every molecule that gets selenium instead of sulfur would be disabled, as it were, and fail to function normally. When a large enough proportion of the molecules are affected, the plant shows external signs of injury. This theory of substitution adequately accounts for the quantitative aspects of the dependence of selenium toxicity on relative rather than absolute sulfur availability; and for the fact that chemical analyses show that excess sulfur reduces the amount of selenium taken up by the plants.

In a series of reports, this same author (Hurd-Karrer, 1934, 1935; Hurd-Karrer and Kennedy, 1936) has cited numerous data which show

that the addition of sulfur in various forms to soils artificially selenized with sodium selenate decreases both the intake of selenium by plants (particularly wheat) and the toxic effects of the plants when fed to rats. Hurd-Karrer considers that a sulfur: selenium ratio of 12:1 or greater insures protection to plants grown on soils artificially selenized with sodium selenate although such wheat grain may be harmful to rats. As might be expected, free sulfur is effective only after time has been allowed for it to change into a soluble form.

Confirmation of the effect of soluble sulfur was obtained by Knight and Beath (1937) who raised wheat upon an originally nonseleniferous soil to which had been added several parts per million of selenium as sodium selenite. To one plot a mixture of sulfur and ammonium sulfate was added to the extent of 0.4 per cent (4,000 p.p.m.). The partly grown plants contained selenium as follows: on sulfur-treated soil, 32 p.p.m.; on untreated soil, 378 p.p.m.

The addition of hexavalent inorganic selenium compounds to soil does not duplicate its natural occurrence either in the original condition or after incorporation in a plant and subsequent return to the earth. Hence the effects of sulfur may be expected to depend upon the condition of the selenium. Hurd-Karrer (1935) found that free sulfur at 1:1,200 in a soil containing 4 p.p.m. of naturally occurring selenium reduced the selenium in plants (presumably wheat) grown on the soil from 450 p.p.m. Se to 15 p.p.m. Byers (1935) made the following cautious statement regarding the effects of sulfur on selenium absorption by range plants: "In general, where the soluble sulfates are high, i.e., the sulfur-selenium ratio is high, the selenium content of comparable samples is low, and, conversely when the sulfur-selenium ratio is low, the plant content is high. However, the relation is not consistent."

In contrast to these two reports, all other workers seem to have agreed that, under practical conditions, sulfur is of no value in decreasing selenium absorption. Martin (1936) reported that only low concentrations of sodium selenite—for example, 1 p.p.m. Se—in nutrient solutions could be rendered nontoxic to wheat plants by adding even large amounts of soluble sulfate. In fact, a S:Se ratio of 2.5:1.0 was as useful as one of 40:1. Franke and Painter (1937) pointed out that Hurd-Karrer's results are inexplicable to many workers familiar with the soils of some regions which produce seleniferous vegetation because these soils are nearly or completely saturated with calcium sulfate and the waters of the areas are rich in soluble sulfate. In their own experiments, no decrease in selenium absorption by corn, wheat, or barley was caused by 1,500 pounds per acre of ground sulfur or calcium sulfate added to a seleniferous soil

one and two years before. During the course of the present investigation, wheat was grown by Hoagland on culture solutions containing 8 p.p.m. Se as sodium selenite and varying concentrations of soluble sulfate. The mature grain was analyzed by Kirk with the following results:

S : Se ratio	Selenium in grain, p.p.m.
2:1 .....	142
4:1 .....	80
10:1 .....	23.4
12:1 .....	42.3

Obviously absorption of selenium was only partly prevented by a 12:1 sulfur : selenium ratio.

Knight and Beath (1937) and Moxon (1937) have arrived independently at the conclusion that sulfur does not inhibit the absorption of the organic combinations of selenium that are given to the soil when "selenium-converter" plants such as *Astragalus* decompose. This was still true when the seleniferous plant had been in the soil for three years. Beath, Eppson, and Gilbert (1937) cite data showing that young wheat plants took in *more* selenium from soil containing powdered *Astragalus* plants when sulfur, sodium sulfate, or magnesium sulfate was added. The foliage of beans grown on a soil plus 0.6 per cent S contained 150 p.p.m. Se, whereas on the same soil in the absence of sulfur, it contained 36 p.p.m. Se.

The large amount of data furnished by the workers mentioned immediately above discourages any hope that the addition of sulfur offers a method for mitigating the harmful effects of naturally occurring selenium in districts where they are serious. Even if the desired effects could be secured by generous use of sulfur, the cost would often be prohibitive.

### TOXICITY OF SELENIUM TO ANIMALS AND MAN

The remarkable story of how a horse, cattle, and hog disease of certain states in the Great Plains and Rocky Mountain regions was traced to ingestion of selenium in the food has been told many times—for example, Trelease and Martin (1936), Steen (1936), Moxon (1937), and Knight (1937).

The distinction between the acute and chronic types of toxic action was recognized long ago. The term "blind staggers" has been applied to the sudden onset of violent symptoms such as impairment of vision, aimless wandering, gnawing of wood and metals, grating of teeth, drooling, grunting, labored breathing, and paralysis ending in death. This condition is the result of eating highly seleniferous plants such as the "selenium converters" and may come on very soon or after a considerable



delay (Beath, 1935; Knight and Beath, 1937). Ordinarily, animals avoid these plants, but in time of drought may be driven to eat them, and animals newly arrived from other localities are not careful to avoid them at any time.

"Alkali disease" signifies the chronic condition characterized by retarded growth of the young, dullness, apathy, emaciation, loss of hair, aged appearance, soreness of joints, and loosening or deformed growth of hoofs. This is the result of continued ingestion of small amounts of selenium, particularly as it occurs in cereals and forage crops.

A detailed summary of the pathologic anatomical changes in both conditions as observed at autopsy has been given by Draize and Beath (1935) and by Smith, Stohlman, and Lillie (1937). Selenium is present in all parts of the body to the extent of several parts per million, especially in the liver, kidney, and spleen (Dudley, 1936). Degenerative changes, such as fatty infiltration and necrosis of the liver and advanced anemia, are more pronounced, of course, in the condition of chronic poisoning.

*Proof that Selenium Is the Cause of the Disorders.*—After suspicion was first aroused that selenium might be the cause of blind staggers and alkali disease, it was necessary to determine if the incidence of the disease corresponded geographically with the occurrence of selenium in plants used for animal food, and to compare the effects of selenium introduced into the body in various forms and ways with the natural symptoms. A review of the very extensive research work which has elucidated these questions is not necessary here, for it has been discussed at length by several writers. Byers (1935), Franke, Rice, *et al.*, (1934), and Knight (1935) have shown convincingly that the area in which selenium occurs to the extent of at least several parts per million in the food of livestock is the same as that in which the disorders occur. Differences between regions are to be expected—for example, alkali disease is the predominant type in South Dakota (Moxon, 1937) where grain is raised very extensively, whereas blind staggers is typical of the range lands of Wyoming.

The attempts to prove that the naturally occurring symptoms are identical with those resulting from experimental feeding of selenium and hence may be attributed solely to that element have resulted in general agreement, although points of difference still persist among various workers. For one thing, experiments have been made with rats, cats, and other small animals, whereas practical interest is in large animals and humans. Beath has written (Knight and Beath, 1937):

The writer has no report of anyone who has produced aggravated cases of the alkali disease in cattle and horses by experimentally controlled feeding tests. Nor has any investigator reported upon the production of the acute stage of blind staggers by

feeding, under controlled conditions, those seleniferous weeds believed to be responsible. . . . So far as the writer can determine, no one has as yet shown that an aggravated case of the alkali disease characterized by deformed hoofs, sloughing of manes and tails, and other loss of hair, can be produced in cattle and horses by daily feeding of the inorganic salts of selenium.

It has been suggested (Beath, Eppson, and Gilbert, 1935) but not proved that other elements, such as tellurium or molybdenum may be concerned in these disorders.

With small experimental animals, the picture is much clearer. On the criteria of growth, food intake, hemoglobin level, and gross pathology of rats, Franke and Potter (1935) decided that the effects of sodium selenite added to a standard diet are "virtually identical" with those produced by the natural plant toxicant, and that for equal intakes of selenium, the toxic effects are quantitatively the same. Schneider (1936) also found that selenite produced the same symptoms in his rats as Franke had described. Martin (1936) compared the effects upon rats of sodium selenite added to a standard Sherman and Campbell diet with those of seleniferous buckwheat and found the toxicities to be identical. The identity of symptoms was corroborated by Munsell, De Vaney, and Kennedy (1936), but these workers found the condition of animals on the diet containing 18.4 p.p.m. selenite selenium to resemble most nearly that of others on toxic wheat diet containing 9.8 p.p.m. Se, that is, the naturally occurring selenium was about twice as toxic as the inorganic form.

In an elaborate summary of nearly ten years' work with rats, Franke and Painter (1938) have declared that on both naturally and artificially selenized diets, the toxic effects are due solely to the presence of selenium. As criteria they chose: average life span, average net gain, difference in net gain between control and experimental, average food consumption and difference in food consumption of control and experimental. For the different sources of the element, the order of decreasing toxicity for diets containing 10 p.p.m. Se or more was wheat, corn, barley, selenite. However, the differences are not great, for example, equal toxicity is associated with the following selenium content in the diets: wheat, 14 p.p.m.; corn, 16 p.p.m.; barley, 18 p.p.m.; selenite, 25 p.p.m. Any difference in toxicity between naturally occurring selenium compounds in plants and selenite is probably due to reduction of the latter to free selenium, which is very poorly absorbed.

*Restriction of Food.*—All workers have noted that rats are loath to eat food containing selenium. Franke and Potter (1936a) found that they invariably chose the food containing the least selenium whether it was present in wheat or as sodium selenite. In the experiments reported by

Miller and Schoening (1938), young pigs were very loath to eat food containing 24.5 p.p.m. Se as sodium selenite. This is entirely analogous to the previously mentioned tendency of animals on the range to avoid seleniferous plants. The question of whether the effects of a diet containing selenium may be due at least in part to the voluntary partial starvation of the test animals has received considerable attention. Symptoms such as diminished growth of the young, loss of weight of the adults, weakness, and general apathy are the usual results of starvation; but the degenerative changes which persist after animals are restored to normal diets are clearly specific results of the ingestion of selenium. Restriction of a normal diet to equal the amount of a seleniferous diet eaten resulted in about equally diminished rates of growth; but the hemorrhages, degenerate liver, and other pathological symptoms were entirely limited to the rats on a diet containing selenium (Munsell, De Vaney, and Kennedy, 1936). The whole matter was examined critically by Franke and Painter (1938) who concluded that: (a) although there is a high correlation between the selenium content of the diet and voluntary food restriction, there is no correlation between the toxic effects produced and the daily consumption of selenium; (b) when much selenium is in the diet, the food intake is reduced to a level which will not maintain life and enable the animal to withstand the toxic action of the selenium; (c) on the diets which caused a diminution in food intake, the rats eating the most selenium fare the best; (d) animals on a seleniferous diet gain less weight per gram of consumed food than they do on normal diets. If this last conclusion holds also for farm animals, tremendous economic losses may be suffered by stockmen in the seleniferous regions entirely aside from the direct losses by illness and death of animals.

*Effects on Reproduction and the Survival of Young Animals.*—A further source of losses of the less obvious kind is that of decreased fertility as a consequence of ingesting selenium. It has long been known that in certain localities of South Dakota unsatisfactory hatching of eggs and malformation and high mortality of chicks are of general occurrence. In a series of studies, Franke and his co-workers (Franke and Tulley, 1935, 1936; Tully and Franke, 1935; Poley, Moxon, and Franke, 1937) showed that these effects are the result of selenium in the chicken feed, and that similar effects may be produced by injecting selenite into the air cell of eggs before incubation. In a feeding experiment in which chickens were placed upon a diet containing 65 per cent seleniferous grain (15 p.p.m. Se), after 7 days no more normal chicks were produced as long as the diet was continued, and the hatchability was reduced to zero in 13 to 19 days; 6 days after removal from the toxic diet these symptoms disappeared.

TABLE 9  
DATA ON ACUTE TOXICITY OF SELENIUM AND OF ARSENIC

Animal	Workers	Method of administering	Form of chemical	Minimum fatal dosage, mg per kg of body weight	Remarks
Selenium					
White rat	{ Muehlberger and Schrenk (1928) . . . . .	Intravenous	{ Selenite Selenate	4.7 4.3	Assuming weight of rat 125 grams  Selenite acts more slowly
	Jones (1909) . . . . .	Hypodermic	Selenite	3.2	
	{ Smith, Franke, and Westfall (1936) Smith, Stohlman, and Lillie (1937) }	Intravenous	{ Selenite Selenate	3.0 3.0	
	{ Franke and Moxon (1936) . . . . .	Intraperitoneal	{ Selenite Selenate	3.25-3.5 5.25-5.7	
Rabbit	{ Muehlberger and Schrenk (1928) . . . . .	Intravenous	{ Selenite Selenate	0.9 1.1	
	{ Smith, Stohlman, and Lillie (1937) . . . . .	Intravenous	{ Selenite Selenate	1.5 2.5	
Cat	Smith, Stohlman, and Lillie (1937) . . . . .	Subcutaneous	Selenite	2-3	Causes vomiting
Dog	Woodruff and Gies (1902) . . . . .	Oral, hypodermic, or rectal	{ Selenite or Selenate Powdered Se	4 4	Fatal in a few minutes 6 grams by mouth had no effect
Arsenic					
White rat -	{ Franke and Moxon (1936) . . . . .	Intraperitoneal	{ Arsenite Arsenate	4.25-4.75 14.0-18.0	
	{ Smith, Franke, and Westfall (1936) . . . . .	Intravenous	Arsenite	5-6	
	{ Muehlberger and Schrenk (1928) . . . . .	Intravenous	{ Arsenite Arsenate	6.0 21.0	
Rabbit	Muehlberger and Schrenk (1928) . . . . .	Intravenous	{ Arsenite Arsenate	6.0 7.0	
Man	McNally (1937) . . . . .	Oral	Arsenite	2.5-3.0	

In a study with rats on diets containing seleniferous wheat, Franke and Potter (1936*b*) showed that prolonged feeding led to a distinct loss in reproductive power. Matings between animals both fed on the toxic grain were invariably sterile; if but one animal had been on the diet, fertility was variable.

Affected females could not raise their young. There is a strong probability that failure of the young to survive was at least partly due to their susceptibility to selenium poisoning as well as to any failure of the mothers to give proper care, for it has been found that selenium occurs to at least 1 p.p.m. in the milk of cows on a seleniferous diet (Smith and Westfall, 1937), and doubtless is present in rat milk under the same circumstances. The hypersensitivity of the young to selenium poisoning is illustrated by the results of Franke and Potter (1936*b*), who found that young rats aged 20–22 days put on a diet of wheat containing 24.6 p.p.m. Se died in a few weeks, whereas those not exposed to the diet until they were 60–100 days old lived through a feeding period of 360 days with no outward signs of injury except a somewhat depressed rate of growth.

There is no proof that similar conditions hold for larger animals, but this work suggests that this and other aspects of chronic selenium ingestion may be of practical significance.

*Quantitative Data on Toxic Effects Due to Selenium.*—Like many other substances which are harmful to animals, the effects caused by selenium vary greatly with such factors as size of dosage, form and manner of administering, and varying susceptibility of different test animals. Distinction between the acute, subacute, and chronic symptoms and dosages is important. It is not always possible to ascertain at what age the animals were used, but, in general, in experiments not involving growth, young adults were used, whereas growth studies usually were started soon after weaning.

The data on acute toxicity refer to the situation in which death follows a single dose within a very few days at the latest. Part of the pertinent data are summarized in table 9. For comparison, certain data on the acute toxicity of arsenic are included.

When death usually ensues but is delayed for a considerable time, the toxicity may be referred to as "subacute." The data in table 10 are for animals kept on a seleniferous diet continuously. In some cases, food consumption has not been reported, and hence no relation between intake of selenium and toxicity can be examined. It will be noted that 1.5 mg Se per kg of body weight daily results in the death of about 30 per cent of the rats after several months on the diet, whereas rabbits and cats are affected at least as severely by 0.3 mg Se per kg of body weight daily or



TABLE 10  
DATA ON SUBACUTE TOXICITY OF SELENIUM

Animal	Workers	Source of selenium	Selenium concentration in diet	Daily dosage per kg. body weight	Remarks
White rat.....	{ Nelson, Hurd-Karrer, and Robinson (1933).....	Wheat	p.p.m. 8-10	mg	Death in a few weeks Per cent dead in 70 days:
			9.9	....	40
			10.7	....	25
			12.2	....	10
			13.1	....	30
	Franke and Painter (1933).....	Corn.....	16.4	....	70
			17.5	....	50
			17.5	....	40
			20.5	....	100
			23.0	....	100
Rabbit.....	{ Martin (1936)..... Trelease and Trelease (1937).....	Selenite or buckwheat	22	....	Majority died in 42 days
			64	....	All died in 5-7 days
			65	....	All died in 4-11 days
			15	1.5	29 per cent died in 30-172 days
			15	1.5	None died in 72-117 days
	{ Smith, Stohlman, and Lillie (1937)..... Smith, Stohlman, and Lillie (1937).....	{ Selenite..... Selenate.....	....	0.3	6 in 9 died after 17-39 doses, 3 survived 113 doses
			....	0.25	2 in 5 died after 60-96 doses, 3 survived 122-150 doses
			....	0.1	1 in 4 died after 40 doses, 3 survived 140 doses
			24.5	0.40 (av.)	Died in 79 days
			....	0.87 (av.)	Died in 46 days
Cat.....	Miller and Schoening (1938).....	Selenite.....	....	....	....
Pig (4 months old)	Smith, Stohlman, and Lillie (1937).....	Selenite.....	....	....	....

less. On the basis of results with only a few animals, the susceptibility of young pigs to subacute poisoning from selenium appears to be between the susceptibilities of white rats and of rabbits and cats.

From the standpoint of public health, the chief consideration in the use of selenium in a spray is the possibility of causing chronic effects which may never result in death but nevertheless may impair general health and efficiency. The data for such conditions, as given by various workers, refer both to selenium content in the food and to actual amounts of selenium ingested. Barley hay containing 6 p.p.m. Se fed to cattle for several months and *Atriplex Nuttallii* (19 p.p.m. Se) fed to cattle and to hogs for 3 months caused no sign of injury (Beath, Eppson, and Gilbert, 1935). Rats on diets containing 1.5 p.p.m. Se in wheat grew normally, reproduced, and reared young fully as successfully as those on the standard diet; with 3 p.p.m. Se in wheat, growth was normal, but reproduction was slightly decreased (Munsell, De Vaney, and Kennedy, 1936). Munsell, De Vaney, and Kennedy (1936) found the addition of selenious acid to the extent of 9.1 p.p.m. Se in the diet of rats caused no abnormalities upon autopsy after 38 weeks. Sodium selenate in the diet at the rate of 7.5 p.p.m. Se did not depress growth of rats, and no pathological symptoms were observable after 6 months (Smith, Stohlman, and Lillie, 1937). As a result of their extensive experience, Franke and Painter (1938) state that any diet containing over 5 p.p.m. Se will retard the growth of rats and one with over 9 p.p.m. will kill rats placed upon it soon after weaning. In experiments conducted by C. D. Leake, of the Pharmacology Division, wheat containing 23.4 p.p.m. Se was found to cause no mortality in young rats within 15 days, but a delay in resuming normal growth persisted for several weeks after the normal diet was restored. This probably indicates that more or less permanent injury had been produced. Probably the conclusion of Byers (1935, 1936), that the tolerance limit is 3-4 p.p.m. Se in all the diet, is a fair summary of the results to date.

The data relating known daily intake of selenium with chronic effects are very scanty. From the information given by Martin (1936), it may be calculated that on a diet containing 12.5 p.p.m. Se in buckwheat the average daily intake of rats was 1.35 mg Se per kg of body weight. Normal growth was obtained on this diet; but when 17.5 p.p.m. Se was present and the daily intake was 1.63 mg Se per kg of body weight, the growth was slightly retarded. In similar experiments with diets containing seleniferous wheat, Munsell, De Vaney, and Kennedy (1936) found the weekly intake of 0.253 mg Se per 100 grams of body weight (0.361 mg Se per kg daily) to result in retarded growth and failure to reproduce. No obvious explanation for the difference in these results is at hand.

*Excretion of Selenium.*—Another method is available for calculating the amounts of selenium ingested by animals, and it is applicable also to humans. Munsell, De Vaney, and Kennedy (1936) found that the amount of selenium stored in a rat's body does not increase indefinitely but reaches the maximum for a given diet in 4 to 8 weeks. Thereafter a balance is maintained if the intake is constant and the animal is not progressively injured. Selenium is excreted in at least three ways—by the urine, feces, and breath. Analyses have shown that the urinary excretion accounts for most of the total loss, for example, 50–80 per cent in the cat (Smith, Westfall, and Stohlman, 1937). Hence, it may be used to calculate a minimum figure for the selenium intake with the probability that the true intake will be higher but not more than twice as great. Dudley (1936) found from 0.1 to 3.0 p.p.m. Se in the urine of hogs which later died as a result of ingesting 15.6 to 74.4 mg Se per kg of body weight daily. A horse was fed 94.0 grams Se as sodium selenite over a period of a year's time; near the end of the feeding period, a sample of urine contained 2.0 p.p.m. selenium; no ill effects of the selenium diet were observed. The data given by Smith, Franke, and Westfall (1936) for urine from several horses suffering from alkali disease are particularly interesting: a colt at autopsy, 0.33 p.p.m.; horse No. 1, 1.00 p.p.m.; horse No. 2, 1.25 p.p.m.; horse No. 3, 1.70 p.p.m.

Valuable information regarding the elimination of selenium after its ingestion has ceased is given by urine analysis. Smith, Westfall, and Stohlman (1937) found that the bulk of stored selenium was eliminated by cats within 2 weeks, but that small amounts occurred in the urine for at least a month longer. Munsell, De Vaney, and Kennedy (1936), by analysis of rat tissues at various times after removal of selenium from the diet, found somewhat comparable conditions with the rat, though depletion was not attained for a longer period.

Smith, Westfall, and Stohlman (1938) have published data on the relative ease of excretion of inorganic and organic selenium by cats and rabbits. Their results show that over feeding periods up to 144 days less than half the ingested selenium from wheat gluten or oats was excreted in the urine. The amounts of selenium stored in the liver and other organs were correspondingly greater than when inorganic selenium was fed. These results were obtained with diets relatively rich in selenium, however, and give no information on the effects of extremely small intake of organic selenium over long periods.

*Chronic Effects of Selenium upon Humans.*—The most important application of the study of urinary secretion of selenium is to the case of humans who live in the regions in which the vegetation is known to be

seleniferous. Smith and co-workers have made two surveys of families in the seleniferous regions of South Dakota, Nebraska, and Wyoming. In the first (Smith, Franke, and Westfall, 1936) the results for 127 individuals in 90 families may be summarized:

Selenium in urine, p.p.m.	Number of persons	Per cent of total
0 .....	4	3.1
trace .....	6	4.8
0.02-0.09 .....	35	27.6
0.10-0.19 .....	22	17.3
0.20-0.49 .....	37	29.2
0.50-0.99 .....	19	14.9
1.00-1.33 .....	4	3.1
TOTAL .....		127
		100.0

The second survey (Smith and Westfall, 1937) covered 100 individuals (including several of the first survey) and showed the urinary selenium to vary from 0.20 to 1.98 p.p.m. Se. The authors have calculated that the persons examined have been absorbing continuously from 0.01 to 0.10, or possibly 0.20 mg Se per kg of body weight per day. This indicates a daily absorption of 1 to 2 mg, and in some cases possibly 5 mg, of selenium for an adult. Since these people had been residing in the same localities for from three to forty years, they were presumably at an equilibrium condition. The conclusion from physical examinations of the subjects was: "Outside of a high incidence of symptoms pointing to gastric or intestinal dysfunction, and a few instances of apparent hepatic dysfunction, both probably the result of continual selenium ingestion, no other evidence of ill health was seen that could be ascribed to selenium with any degree of certainty."

To gain information regarding the sources of the selenium to which persons included in these surveys were exposed, analyses were made of several foods. The results in brief were:

Food	Number of samples	Selenium, p.p.m.
Canned or salted pork .....	4	1.17 to 8.00
Raw chicken muscle .....	1	2.19
Lean cooked beef .....	1	2.22
Milk .....	50	0.16 to 1.27
Eggs .....	32	0.25 to 9.14
Grains .....	42	0.87 to 18.80
Vegetables .....	95	0.00 to 17.80

A study of the relation between selenium content of the various food-stuffs and urinary elimination proved that the chief sources are meats, eggs, milk, and grains. Vegetables played little part, but since they were

scarce during the drought year of 1936, this conclusion may not be true in general.

For comparison, the data given by Byers (1935) and by Dudley and Byers (1935) show from 0.02 to 3.00 p.p.m. Se in whole milk from the same general region and 9.6 p.p.m. Se in the milk solids.

## DISCUSSION AND CONCLUSIONS

The attention of the medical profession, of scientific and popular writers, of governmental agencies, and of the general public has been aroused during recent years to the health hazard arising from the use of various poisonous substances for the protection of plants which are used as foods. Entomologists and agricultural chemists are well aware of the situation, and a truly enormous effort is being made to eliminate such toxic elements as lead, arsenic, etc., from agricultural use and to substitute organic substances in their place. As yet, however, no more than a beginning has been made. In the face of this feeling against inorganic materials, the advisability of using such a product must be considered with particular care. If it offers a menace to public health, its use should be abandoned if possible and carefully controlled in any event. The two criteria are: how poisonous is the substance in question and how much of it will be present in food as a result of its use for control of pests? The foregoing lengthy account of work in this and other laboratories is intended to be a summary of the pertinent available information from which conclusions may be drawn regarding the use of the selenium-containing product Selocide, particularly on citrus trees and grapevines.

There is almost no conceivable danger of acute poisoning from eating citrus or grapes treated with Selocide, for it may be estimated from the data given that the amount of selenium needed is several hundred milligrams, which would be furnished by half a ton or more of oranges or grapes. The real question is concerned with the cumulative effect of daily ingestion of small amounts of selenium. The toxicity data indicate that for several animals the safe limit, that is, the amount which produces no detectable chronic symptoms, is about 3 p.p.m. Se in the whole diet. It appears that this figure may be used for humans also since the food of the families in the seleniferous regions contained at least this much selenium on the average and no definite symptoms attributable with certainty to selenium were found in the group. There is no decided difference in the toxicity of inorganic and organic selenium, except that the free element has very little effect, owing in part to restricted absorption from the digestive tract. If the use of Selocide resulted in the high concentration of 0.2 p.p.m. Se in the fruit and if it is all absorbed during digestion,



a simple calculation shows that in order to ingest 1 mg of selenium per day, a person must eat about 11 pounds of the contaminated product daily. The results of Smith, Westfall, and Stohlman (1937) indicate that 1 mg of selenium per day is not harmful to an adult. Even though children, like young rats, probably are more sensitive to selenium than adults, a very large amount of contaminated fruit would be necessary to exert a harmful effect. Of course, if selenium is present in other constituents of the diet, an additional intake from any source may cause the total intake to reach or exceed the safe limit, but in that case, the rest of the diet usually is the greater source of danger. The special case of by-products in which selenium might become concentrated is a matter which the industries concerned must attend with especial care, just as also must be done in the cases in which lead or arsenic are involved.

The subacute and chronic effects of selenium upon lower animals have been shown to be due in part to voluntary decrease in intake of seleniferous food. In the case of human diets, only parts of which contain appreciable amounts of selenium, it is reasonable to expect this effect of semistarvation to be diminished or eliminated. Hence, under conditions of choice of food, the results of daily ingesting a given amount of selenium should be less than when all the food is seleniferous. It does not seem likely that the amounts of selenium which may occur in human foods would lead to avoidance of such foods, for Franke and Potter (1936a) found that rats made but little distinction between control grain and that containing 6.15 p.p.m. Se, though the young ones were very reluctant to eat grain containing 12.3 p.p.m. Se.

In order to remove the uncertainty of agriculturists regarding possible legal complications from the use of selenium, it is desirable that a tolerance be set. The indications are that 3 p.p.m. Se would be entirely safe, but a lower figure would probably work no hardship and would be a stronger guarantee to the public that their food was fit to eat.

Although selenium occurs in large amounts in only a few plant species, the indication is that all plants take it up to some extent. Hence the element is not a stranger to the diet of animals or man, and the intake required to produce toxic effects is probably the result of a physiological tolerance acquired by exposure through the ages. There seems to be no reason to hope that this natural partial immunity to selenium can be increased rapidly: Smith, Stohlman, and Lillie (1937) found no evidence of such an effect with cats.

The analyses discussed in a previous section show that the amount of selenium added to a diet as the result of its use on citrus is extremely minute. The residue on the outside is in the least toxic (elementary) form

and ordinarily would not be eaten. Furthermore, nearly all of it is removed during the regular packing-house treatment. Most of that which occurs in the interior of the fruit will ordinarily be ingested, though there is some evidence that in other plants the seeds contain more selenium than the other parts. Inspection of the data shows that over a period of five years' use there is only a very minute increase in the amount of selenium in the interior of citrus fruits. This is to be expected from the behavior of the selenium which reaches the soil as the result of the spraying process, for most of it remains in the first 6 inches and practically none has reached beyond the first 2 feet. Doubtless longer use will eventually result in deeper penetration so that more selenium will reach the feeding zone of citrus roots. Whether or not this will result in a considerable increase in absorption depends to a large extent upon the rainfall and irrigation practice.

Rainfall in the citrus districts of California varies considerably but may be taken as 15 inches on the average. Irrigation adds many more inches of water each year in most of the citrus districts. However, a considerable part of the irrigation water used up to the present time is obtained from wells in the various districts and hence is recirculated water. This condition is not so favorable for removal of soluble selenium as prevails in localities such as the Belle Fourche district of South Dakota (Byers, 1935), in which much of the water leaches through the soil and flows away by gravity. The introduction of irrigation water from the outside would increase the probability that soluble selenium will remain at a low level in the soil of the citrus district in southern California.

In the case of grapes, conditions are not so favorable for the use of selenium sprays. In the first place, the residue is greater and there is less chance to remove it, particularly from fresh fruit. Proper washing before drying should keep the selenium residue on raisins down to a low figure. The assimilation of selenium from the soil by grapes appears to be somewhat greater than in the case of citrus, but the analyses have all been made on grapes from the same district and conditions may be different elsewhere. Another factor to be considered is the probability that vineyards will be torn out in the course of time and the land used for other crops. Again in this case, the amount of water received by the soil is a very important factor; probably the normal rainfall or rainfall together with necessary irrigation is sufficient to prevent any serious accumulation of selenium as the result of the proper use of Selocide.

## SUMMARY

A spray made by dissolving selenium in a solution of potassium ammonium sulfide in such proportions that the composition corresponds to the empirical formula  $(K NH_4S)_3Se$  is effective for the control of mites on citrus and grapes. After several years' use, there is no evidence of harmful effects upon the plants, and fruit injury has occurred to only a limited extent in certain districts.

Analyses of citrus fruits has shown that the residue, which is mainly free selenium, is present to the extent of two to practically zero parts per million *in the rind*, according to the length of time between the application of the spray and the analysis. Analyses of the soil beneath sprayed trees showed that, after five years' treatment, a maximum of two parts per million selenium occurred in the first 6 inches, but penetration to lower depths was very slow. Absorption from the soil resulted in only a few hundredths part per million selenium in the interior of citrus fruits.

The residue upon grapes is somewhat greater on account of the larger surface which they present to the spray, and the analytical data indicate that they absorb somewhat more selenium from the soil.

The data regarding the acute, subacute, and chronic toxic effects of ingested selenium have been reviewed. There is considerable evidence that three parts per million selenium in the whole diet causes no symptoms of injury. Such an amount could not be obtained from citrus fruit or grapes. Hence it is concluded that proper use of the selenium-containing spray mentioned above on citrus and grapes, under the prevalent conditions of production in California, offers no hazard to public health.

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